Disruption of γ-glutamylcysteine synthetase results in absolute glutathione auxotrophy and apoptosis in *Candida albicans*

Yong-Un Baek, Yeon-Ran Kim[1], Hyung-Soon Yim*, Sa-Ouk Kang*

Laboratory of Biophysics, School of Biological Sciences, and Institute of Microbiology, Seoul National University, Seoul 151-742, South Korea

Received 9 September 2003; revised 3 November 2003; accepted 12 November 2003

First published online 27 November 2003

Edited by Giovanni Cesareni

Abstract Glutathione is the most abundant non-protein thiol and a major source of reducing equivalents in eukaryotes. We examined the role of glutathione in *Candida albicans* by the disruption of γ-glutamylcysteine synthetase (GCS1), an essential enzyme in glutathione biosynthesis. The *gcs1*/*gcs1* null mutants exhibited glutathione auxotrophy, which could be rescued by supplementing with reduced and oxidized glutathione and γ-glutamylcysteine. When the mutants were depleted of glutathione, they showed typical markers of apoptosis. These results suggest that glutathione itself is an essential metabolite and *C. albicans* lacking GCS1 undergoes apoptosis.

Key words: Glutathione; γ-Glutamylcysteine synthetase; Apoptosis; *Candida albicans*

1. Introduction

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) is the most abundant intracellular thiol in eukaryotic cells and is known to be involved in many biological processes, including the synthesis of proteins and DNA, transport, enzyme activity modulation, and metabolism as well as defense against reactive oxygen species (ROS). GSH is synthesized in two steps by γ-glutamylcysteine synthetase (GCS) and glutathione synthetase. The first step catalyzed by GCS has been known to be the rate-limiting step and feedback inhibited by GSH [1]. Null mutations of the gene encoding GCS in *Saccharomyces cerevisiae* (*GSH1*) and *Schizosaccharomyces pombe* (*gcs1*) result in GSH auxotrophy, implying essential requirement of GSH in yeast [2–5]. In mammals, disruption of GCS leads to the embryonic lethality and blastocysts from such knock-out strains fail to grow, except in the presence of GSH or *N*-acetylcysteine, the GSH substitute [6]. However, the reason for the essential requirement of GSH in eukaryotes is not known. In contrast, the GCS (*gshA*) mutants of *Escherichia coli* have nearly wild-type growth rates in rich and minimal media [7–9], indicating that GSH is dispensable for normal growth of *E. coli*. Moreover, only the simultaneous inactivation of both the GSH and the thioredoxin pathways results in aerobic inviability due to defects in protein thiol maintenance and/or ribonucleotide reduction [10,11].

Candida albicans is a well-known opportunistic fungal pathogen of humans that does not usually cause disease in immunocompetent hosts but causes serious diseases in immunocompromised patients such as those suffering from AIDS or undergoing chemotherapy [12]. Once *C. albicans* has infected a host, it inevitably encounters ROS generated by host phagocytes as well as those produced through its own metabolism [13]. Thus the antioxidant defense systems of *C. albicans* were thought to be essential for this organism to resist the host immune response and to exhibit full virulence as demonstrated by the facts that erythroascorbic acid-deficient [14] or copper- and zinc-containing superoxide dismutase-deficient *C. albicans* strain [15] showed defective hyphal growth and attenuated virulence. In addition, mitochondrial manganese-containing superoxide dismutase has been shown to be required for *C. albicans* to resist various external stresses, such as redox-cycling agents, high concentration of salts, ethanol or hyperoxic conditions [16]. From the results for the other antioxidant defense systems mentioned above, GSH is thought to play a role in the ability of *C. albicans* to defend itself against ROS and grow in the host successfully.

In the present study, the functional roles of GSH are investigated by observing the effects of disruption of the *GCS1* gene encoding GCS in *C. albicans* and the resulting apoptosis of *C. albicans* are reported.

2. Materials and methods

2.1. C. albicans *strains and culture conditions*

C. albicans strains used in this study are listed in Table 1. For routine growth of *C. albicans* cells, YPD medium (1% yeast extract, 2% peptone, and 2% glucose) was used. Cells carrying plasmids or disrupted gene were cultured in minimal defined medium containing 0.67% yeast nitrogen base without amino acids (Difco), 2% glucose and appropriate supplements. Solid media were prepared by adding 1.8% agar to liquid broth. All cultures were incubated at 28°C.

2.2. *Isolation, subcloning and sequencing of* GCS1 *from* C. albicans

To isolate *GCS1* gene from *C. albicans*, the degenerate oligonucleotide primers corresponding to conserved residues FHPEYG and DFENAAY of several sources were synthesized; 5′-TTTCAYCCWGARTAYGG-3′ (GCSP1) and 5′-TADGCDGCRTTYTCRAARTC-3′ (GCSP2), respectively, where Y = C, T; W = A, T; D = G, A, T; and R = A, G. Polymerase chain reaction (PCR) amplification was carried

*Corresponding authors. Fax: (82)-2-888 4911.
E-mail addresses: wuseok@hotmail.com (H.-S. Yim), kangsaou@snu.ac.kr (S.-O. Kang).

[1] Present address: Department of Biotechnology, Juseong College, Chung-Buk 363-794, South Korea.

Abbreviations: GSH, glutathione; GSSG, glutathione disulfide; ROS, reactive oxygen species; FACS, fluorescence-activated cell sorter; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01363-2

Table 1
C. albicans strains used in this study

Strain	Genotype	Source or reference
ATCC 10231	Wild-type isolate	ATCC[a]
SC5314	Wild-type isolate	Fonzi and Irwin [17]
CAI4	*Δura3::imm434/Δura3::imm434*	Fonzi and Irwin [17]
YB201	*Δura3::imm434/Δura3::imm434Δgcs1::hisG-URA3-hisG/GCS1*	This work
YB202	*Δura3::imm434/Δura3::imm434 Δgcs1::hisG/GCS1*	This work
YB203	*Δura3::imm434/Δura3::imm434Δgcs1::hisG/Δgcs1::hph-URA3-hph*	This work
YB204	*Δura3::imm434/Δura3::imm434 Δgcs1::hisG/Δgcs1::hph*	This work
YB204R	*Δgcs1::hisG/Δgcs1::hph::GCS1::URA3*	This work

[a]American Type Culture Collection.

out for 30 cycles under the following conditions: denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min. Among the amplified DNA fragments, a 1.1-kb fragment was cloned into pGEM-T easy vector (Promega). The insert DNA fragment was labeled with digoxigenin (Boehringer Mannheim) and used as a probe to screen the *C. albicans* genomic library [14]. Three positive clones were selected and the common 4.5-kb *Cla*I/*Kpn*I fragment was isolated and cloned into pGEM-7Zf(+) at the *Cla*I/*Kpn*I site, yielding pGCS. The sequencing of the cloned DNA was carried out by dideoxy chain termination method by an automatic sequencer (ALFexpress, Amersham Pharmacia Biotech).

2.3. Disruption and reintegration of C. albicans GCS1

Disruption of *GCS1* in *C. albicans* was carried out as described by Fonzi and Irwin [17] with some modifications. First, a 4.0-kb *Bgl*II/*Bam*HI fragment containing *hisG-URA3-hisG* gene disruption cassette was isolated from p5921 [17] and ligated with *Eco*RV/*Bgl*II-digested pGCS, yielding pYB201. The yielded pYB201 was cut with *Apa*I/*Sac*I and the resulting 6.5-kb fragment was used for transformation into CAI4. Ura$^+$ transformants were selected on uracil-deficient medium, and integration of the cassette into the *GCS1* locus was verified by either PCR or Southern blot analysis. Spontaneous Ura$^-$ derivatives of the heterozygous disruptants were selected on minimal defined medium (FOA medium) containing 5-fluoroorotic acid (625 μg ml^{-1}) and uridine (100 μg ml^{-1}). Next, we constructed a plasmid containing gene disruption cassette that can be excised with *Sac*I/*Hin*dIII. A 0.51-kb PCR-amplified fragment corresponding to 156–687 nucleotides of *GCS1* and a 0.58-kb PCR-amplified fragment corresponding to 1458–2055 nucleotides of *GCS1* were consecutively cloned into pQF86 [18], which had the *hph-URA3-hph* gene disruption cassette at *Sac*I/*Kpn*I and *Sal*I/*Hin*dIII sites, respectively, yielding pYB202. The yielded plasmid, pYB202 was digested with *Sac*I/*Hin*dIII and the resulting fragment was used for transformation into one copy disruptants of *GCS1* (YB202). Ura$^+$ transformants were selected on uracil-deficient medium containing 1 mM GSH, and integration of the cassette into the *GCS1* locus was verified by genomic PCR and Southern blot analysis. Primers used to verify disruption were as follows: 5′-GGTACACCTTTAAGTTGGG-3′ (GCSP3), 5′-GGAACTCTTTGAATTGGTTTACC-3′ (GCSP4), and 5′-GGTTGGTATTTCCCCATTGG-3′ (GCSP5), respectively. For reintegration of the *GCS1* gene into the genome, an *Xba*I/*Apa*I-digested *URA3* fragment from pURA3 [14] was inserted into the *Xba*I/*Apa*I sites of pGCS to yield pYB203. pYB203 was linearized at the unique *Eco*RV site within the *GCS1* coding region and used to integrate into the *GCS1* locus in the Ura$^-$*gcs1*/*gcs1* strain. The targeted reintegration of *GCS1* into the genome was verified by viability of the transformants on minimal defined medium without GSH.

2.4. Growth of C. albicans in the presence of various thiol compounds

To monitor the effect of various thiols on growth, *C. albicans* cells were spotted on minimal defined medium containing GSH, glutathione disulfide (GSSG), γ-glutamylcysteine, cysteine, *N*-acetylcysteine, dithiothreitol, methionine, respectively. Plates were incubated for 3 days at 28°C.

2.5. Fluorescence-activated cell sorter (FACS) analysis of C. albicans by annexin-V staining

Exposed phosphatidylserine was detected by reaction with FITC (fluorescein-5-isothiocyanate)-coupled annexin-V (annexin-v-fluos staining kit, Boehringer Mannheim). Cells cultured for 2 days in minimal defined medium at 28°C were harvested and washed in sorbitol buffer (1.0 M sorbitol, 0.1 M EDTA, pH 7.5), digested with lyticase for 2 h at 28°C. The spheroplasts were resuspended in 100 μl of staining solution (annexin-V-fluorescein labeling reagent:propidium iodide = 1:1 (v/v)) and incubated for 15 min at room temperature. Exposed phosphatidylserine was measured by FACS (FACStar plus, Becton Dickinson).

2.6. Detection of DNA fragmentation by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

For the TUNEL test, DNA ends were labeled using an in situ cell death detection kit (Boehringer Mannheim). Cells fixed with 3.7% formaldehyde were digested with lyticase and incubated in permeabilization solution (0.1% Triton X-100 and 0.1% sodium citrate) for 2 min on ice. The spheroplasts were washed with phosphate-buffered saline and resuspended in 50 μl of TUNEL reaction mixture for 1 h at 37°C. DNA fragmentation was observed under a fluorescence microscope.

2.7. Measurement of ROS production

Cells (2×10^7/ml) cultured for 2 days were preincubated in fresh minimal defined medium with 20 μM dicholorodihydrofluorescein diacetate at 30°C for 1 h [19]. Intracellular ROS production was measured by a fluorescence spectrophotometer with excitation at 480 nm and emission at 530 nm (Model 4800S, SLM Instrument).

2.8. Nucleotide sequence accession number

The nucleotide sequence of *GCS1* from *C. albicans* ATCC 10231 has been deposited in the GenBank database under accession number AF176677.

3. Results

3.1. Isolation and characterization of GCS1 in C. albicans

From the comparison of the predicted amino acid sequence of GCS from several sources, two highly conserved regions were identified. PCR using the oligonucleotide primer pair corresponding to residues FHPEYG and DFENAAY of GCS could amplify a DNA fragment of 1137 bp from the chromosomal DNA of *C. albicans*. When cloned and sequenced, the fragment showed a high degree of amino acid sequence similarity to *S. cerevisiae* and *S. pombe* GCS upon BLAST searches of the GenBank database. The cloned PCR product was used as probe to screen the λEMBL3 genomic library. From positive clones, the 4.5-kb *Cla*I/*Kpn*I fragment was subcloned in pGEM-7Zf(+) and sequenced.

The sequenced insert contained a continuous open reading frame of 2070 bp that encoded a polypeptide consisting of 690 amino acids with a calculated molecular mass of 79 190 Da. The deduced amino acid sequence of GCS from *C. albicans* was 50%, 46%, and 46% identical to those from *S. cerevisiae*, *S. pombe*, and human, respectively.

3.2. Disruption of GCS1 in C. albicans

An in vitro construct, plasmid pYB201, was prepared by replacing a portion of the coding region of *GCS1* with the

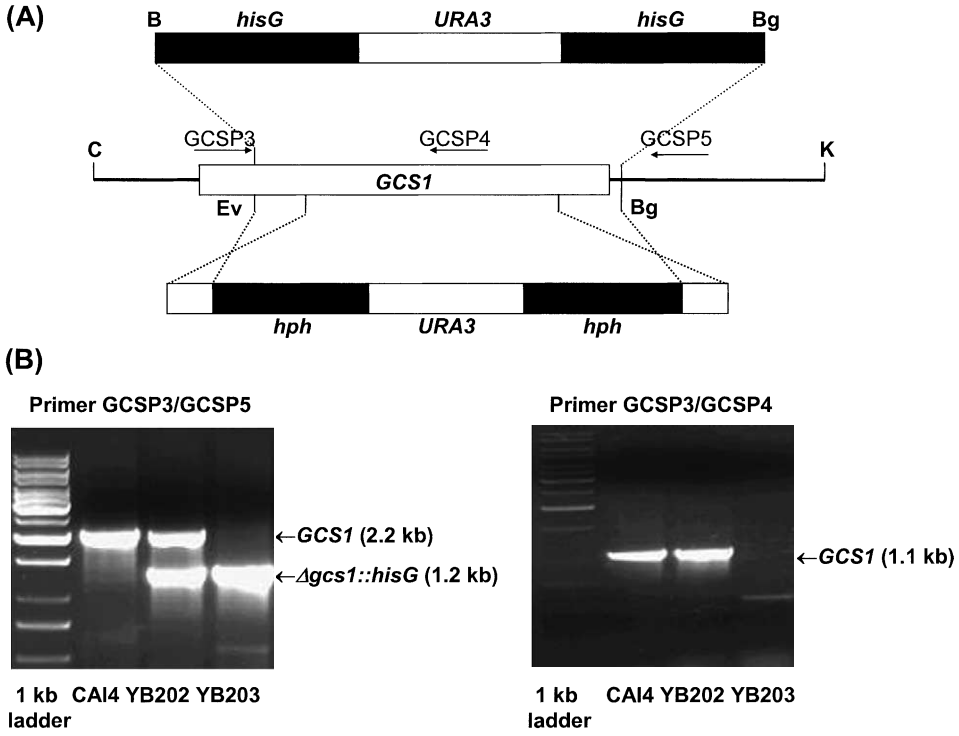

Fig. 1. Disruption of *GCS1* in *C. albicans*. A: Restriction map of the *GCS1* locus and sequential insertion of the *hisG-URA3-hisG* cassette and *hph-URA3-hph* cassette in the *GCS1* coding sequence. The endonuclease restriction sites are as follows: C, *Cla*I; Ev, *Eco*RV; K, *Kpn*I; Bg, *Bgl*II; B, *Bam*HI. B: PCR analysis demonstrating generation of a *gcs1/gcs1* mutant with GCSP3/GCSP5, GCSP3/GCSP4 used as primer pairs; CAI4 (*GCS1/GCS1*), YB202 (*Δgcs1::hisG/GCS1*), YB203 (*Δgcs1::hisG/Δgcs1::hph-URA3-hph*).

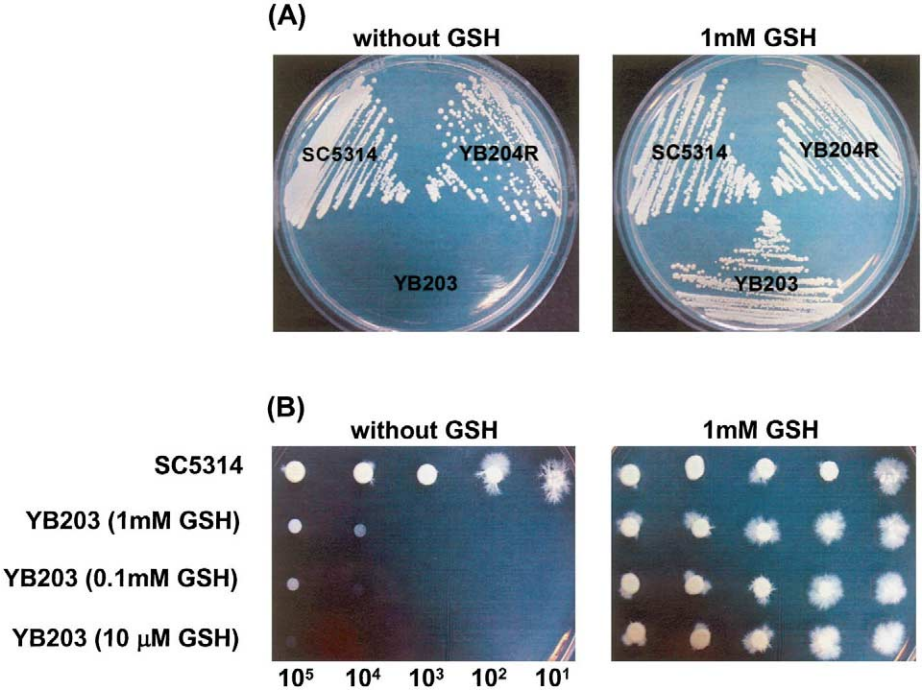

Fig. 2. Absolute requirement of exogenous GSH of *gcs1/gcs1* null mutants for growth. A: Each strain was streaked on minimal defined medium with 1 mM GSH or without GSH. Plates were incubated for 3 days at 28°C. B: Each strain was grown with the indicated GSH concentration (YB203) or without GSH (SC5314) in minimal defined medium to saturation, respectively, diluted appropriately, spotted on minimal defined medium and incubated for 3 days at 28°C in an anaerobic jar flushed with nitrogen gas; SC5314 (*GCS1/GCS1*), YB203 (*gcs1/gcs1*), YB204R (*gcs1/gcs1/GCS1*).

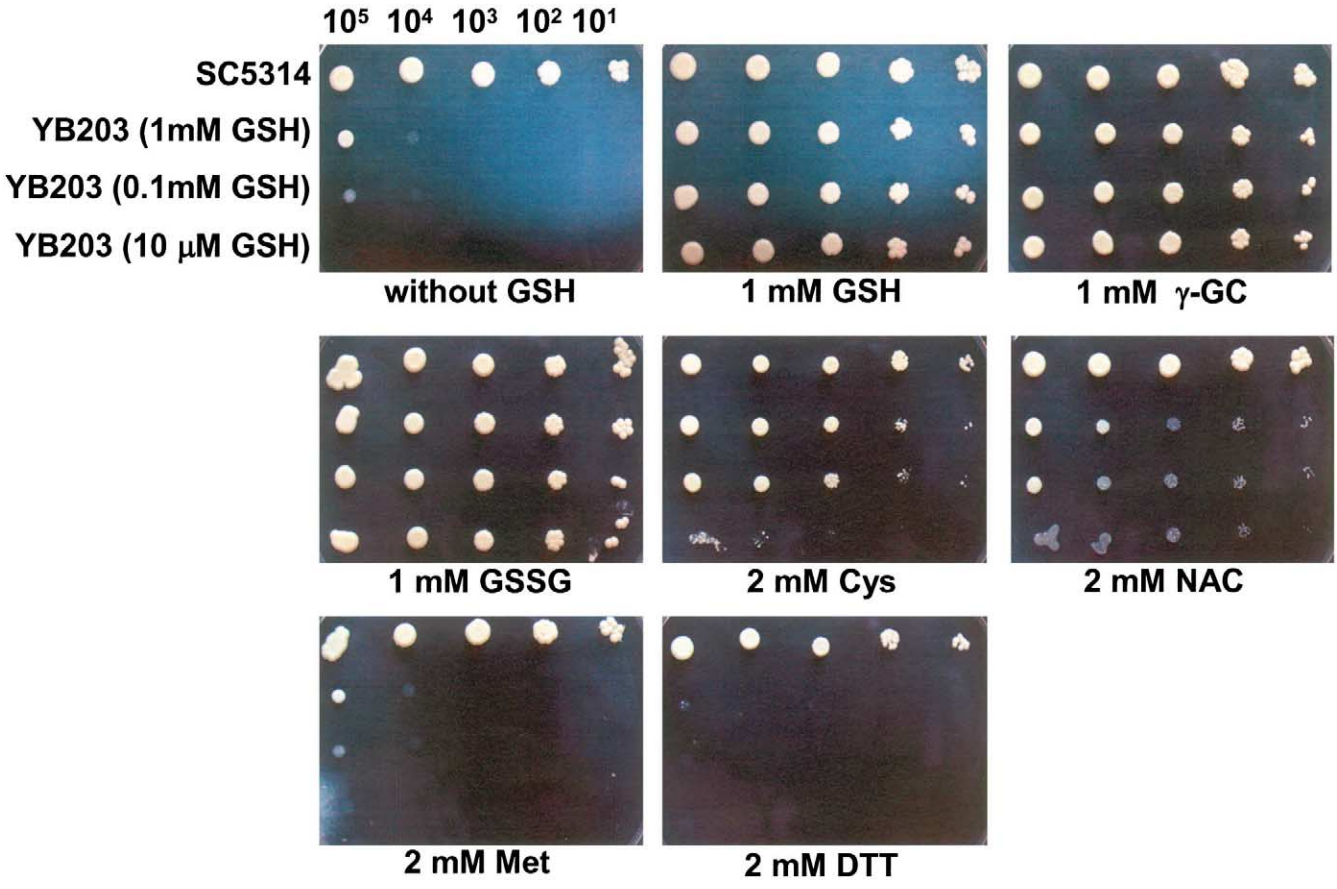

Fig. 3. Effect of exogenous thiols on the growth of *gcs1*/*gcs1* null mutants. Each strain was grown with the indicated GSH concentration (YB203) or without GSH (SC5314) in minimal defined medium to saturation, respectively, diluted appropriately, and spotted on minimal defined medium containing various thiols. Plates were incubated for 3 days at 28°C; SC5314 (*GCS1*/*GCS1*), YB203 (*gcs1*/*gcs1*).

hisG-URA3-hisG cassette (Fig. 1A). Digestion of pYB201 with *Apa*I/*Sac*I released a 6.5-kb fragment, which was used to transform CAI4. The resulting Ura$^+$ transformants were screened by either PCR or Southern blot analysis (Fig. 1B). Spontaneous Ura$^-$ derivatives of the heterozygous disruptants were selected on FOA medium. Next round of disruption was done using pYB202, which had the *hph-URA3-hph* cassette, and Ura$^+$ transformants were selected on uracil-deficient medium containing 1 mM GSH, since *gcs1*/*gcs1* mutants did not grow in the absence of GSH.

3.3. *Essential requirement of* GCS1 *for the growth of* C. albicans

To understand the role of *GCS1* in *C. albicans*, the growth of the disruptant strain (YB203) was monitored. The *gcs1*/*gcs1* mutants were non-viable in minimal defined medium (Fig. 2A). In addition, the growth of the mutants could not be recovered by incubation under anaerobic condition (Fig. 2B). The growth of the mutants could be recovered by the supplementation of GSH, GSSG, and γ-glutamylcysteine in the medium, respectively (Fig. 3). However, the disruptants failed to grow in the GSH-free medium and showed restricted growth in the medium containing *N*-acetylcysteine or cysteine after preincubation with GSH-containing media, indicating GSH itself not the functions of GSH is required for the growth of *C. albicans*.

3.4. *Disruption of* GCS1 *results in increased ROS production and apoptosis in* C. albicans

To examine whether depletion of GSH could induce apoptotic phenotype in *C. albicans* as previously reported in *S. cerevisiae* [20–24], the *gcs1*/*gcs1* mutants grown in GSH-containing medium were transferred to the minimal medium without GSH. The mutants showed membrane staining with annexin-V, indicating an exposure of phosphatidylserine at the outer layer of the cytoplasmic membrane (Fig. 4A). They also showed DNA fragmentation as visualized by TUNEL staining (Fig. 4B), suggesting that *gcs1*/*gcs1* mutants died of apoptosis. Moreover, cellular ROS production was increased up to 1.75-fold in the mutants in comparison with wild-type (Fig. 4C) when measured by oxidation of dichlorodihydrofluorescein diacetate.

4. Discussion

In general, GSH is known to be an essential metabolite in eukaryotes [2–5,25,26]. Disruption of the genes encoding GCS result in GSH auxotrophy from yeasts to mammals. In contrast, GSH is not essential in prokaryotes since strains of *E. coli* lacking *gshA* can grow in minimal media without GSH supplementation [7–9].

The *gcs1*/*gcs1* null mutants showed GSH auxotrophy like other eukaryotes reported so far. In order to test whether

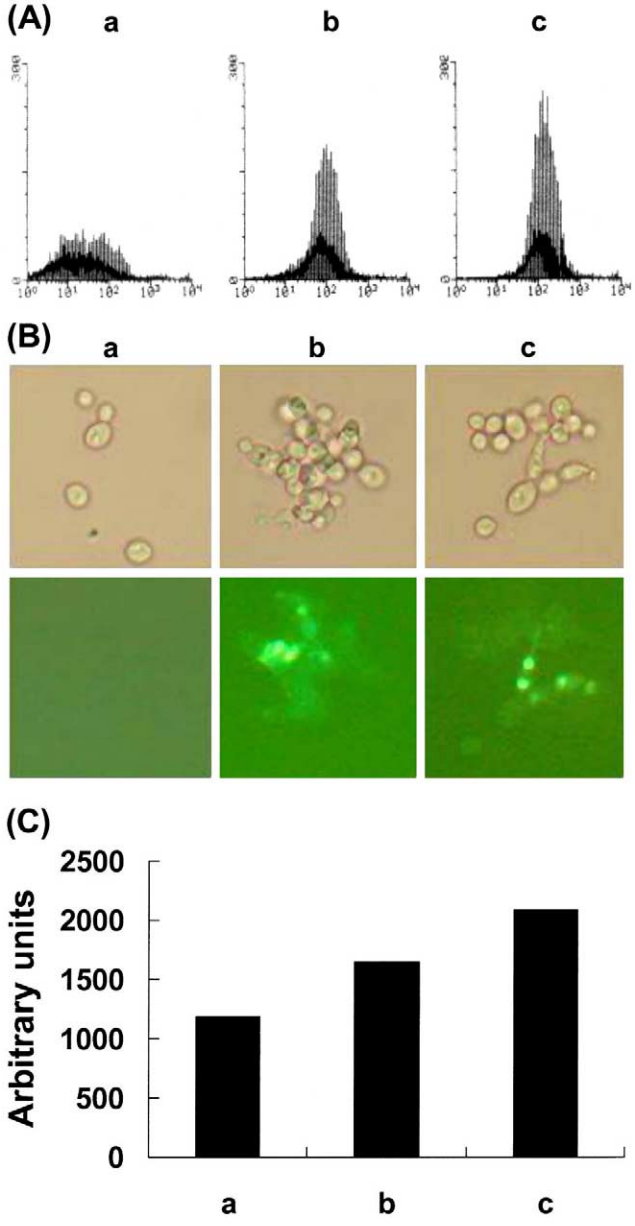

Fig. 4. Analysis of markers of apoptosis and ROS production in *gcs1*/*gcs1* null mutants. A: FACS analysis of *C. albicans* strains by annexin-v-fluos. B: DNA strand breakage visualized by TUNEL staining. C: Increased ROS generation in the *gcs1*/*gcs1* mutants. *C. albicans* cells grown to saturation with 1 mM GSH (YB203) or without GSH (SC5314) were inoculated to 1 mM GSH (YB203, b) or GSH-free medium (SC5314, a; YB203, c) and after 2 days they were subjected to FACS analysis after annexin-V staining, stained with the TUNEL assay kit, and ROS measurement, respectively. TUNEL-stained cells were observed under a fluorescence microscope; SC5314 (*GCS1*/*GCS1*), YB203 (*gcs1*/*gcs1*).

exogenous thiol compounds could recover GSH auxotrophy, the null mutants were incubated in the presence of various thiol compounds in minimal defined medium. Among the tested thiols, only GSH, GSSG, and γ-glutamylcysteine, which can be converted into GSH, could support normal growth of the mutants. These results are in contrast to those from other eukaryotes such as *S. cerevisiae* and mouse, in which GSH auxotrophy could be overcome by thiol reductants dithiothreitol, cysteine, β-mercaptoethanol, and *N*-acetylcysteine [2,26,6]. In addition, anaerobic conditions did not rescue the GSH auxotrophy of the mutants, which suggests that the essential requirement of GSH is probably not related to oxidative stress or to toxic accumulation of non-native protein disulfides. The GSH auxotrophy does not seem to be due to a defect in sulfate assimilation since methionine and cysteine cannot rescue the growth defect of the *gcs1*/*gcs1* mutants. The fact that GSH auxotrophy of *gcs1*/*gcs1* mutants was suppressed exclusively by GSH suggests that GSH itself is essential for the growth of *C. albicans*.

Finally, the *gcs1*/*gcs1* mutants depleted of GSH showed typical markers of apoptosis characterized by an exposure of phosphatidylserine at the outer layer of the cytoplasmic membrane and DNA strand breakage. Moreover, increased ROS generation was observed in the mutants. Apoptosis is the most frequently encountered form of programmed cell death in higher eukaryotes and has been assumed unique to multicellular animals. For a unicellular organism like yeast, a suicide mechanism seemed to be useless. But unexpectedly, expression of some proapoptotic genes including Bax [27], caspases [28] and Apaf-1/CED-4 [29] killed yeast. ROS are a well-characterized class of apoptotic inducers in mammalian cells [30–33]. They have been known to be involved in activation of various redox-sensitive signaling pathways, which can lead to apoptosis, including transcription factors NF-κB, p53, and c-Jun amino-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), the phosphoinositide 3-kinase PI(3)K/Akt pathways [34–37]. In *S. cerevisiae*, exposure to low doses of H_2O_2 or increased radical stress by depletion of GSH induces apoptosis as well [24,23,21]. Recently a caspase which is activated by H_2O_2 or aging was identified for the first time in *S. cerevisiae* [38], confirming the yeast programmed cell death.

In the present study, the *gcs1*/*gcs1* mutants depleted of GSH showed similar apoptotic phenotype as in yeast accompanied by an accumulation of ROS. From these results it is proposed that apoptosis-like features in yeast also exist in *C. albicans* and increased ROS generation plays a crucial role in the induction of apoptosis. It is plausible that redox state unbalance due to depletion of GSH, which can influence phosphorylation cascades and/or modification of reactive cysteines in proteins involved in yet unidentified apoptotic pathways, eventually results in apoptosis in *C. albicans*. This is the first report that *C. albicans* undergoes apoptosis that can be induced by depletion of GSH. Further investigations about the nature of apoptotic features and the relationship between GSH, ROS and apoptosis in *C. albicans* are required.

In conclusion, our results suggest that GSH is indispensable to the growth of *C. albicans*, and is involved in apoptosis in *C. albicans*.

Acknowledgements: We thank W.A. Fonzi, M. Whiteway and G.R. Fink for providing *C. albicans* strains and plasmids. This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (00-PJ1-PG3-20200-0036) and by Research Fellowship of the BK21 project.

References

[1] Meister, A. and Anderson, M.E. (1983) Annu. Rev. Biochem. 52, 711–760.
[2] Grant, C.M., MacIver, F.H. and Dawes, I.W. (1996) Curr. Genet. 29, 511–515.
[3] Ohtake, Y., Satou, A. and Yabuuchi, S. (1990) Agric. Biol. Chem. 54, 3145–3150.

[4] Wu, A.L. and Moye-Rowley, W.S. (1994) Mol. Cell. Biol. 14, 5832–5839.
[5] Chaudhuri, B., Ingavale, S. and Bachhawat, A.K. (1997) Genetics 145, 75–83.
[6] Shi, Z.-Z., Osei-Frimpong, J., Kala, G., Kala, S.V., Barrios, R.J., Habib, G.M., Lukin, D.J., Danney, C.M., Matzuk, M.M. and Lieberman, M.W. (2000) Proc. Natl. Acad. Sci. USA 97, 5101–5106.
[7] Apontoweil, P. and Berends, W. (1975) Biochim. Biophys. Acta 399, 10–22.
[8] Greenberg, J.T. and Demple, B. (1986) J. Bacteriol. 168, 1026–1029.
[9] Fuchs, J.A. and Warner, H.R. (1975) J. Bacteriol. 124, 140–148.
[10] Prinz, W.A., Aslund, F., Holmgren, A. and Beckwith, J. (1997) J. Biol. Chem. 272, 15661–15667.
[11] Stewart, E.J., Aslund, F. and Beckwith, J. (1998) EMBO J. 17, 5543–5550.
[12] Cutler, J.E. (1991) Annu. Rev. Microbiol. 45, 187–218.
[13] Vázquez-Torres, A. and Balish, E. (1997) Microbiol. Mol. Biol. Rev. 61, 170–192.
[14] Huh, W.-K., Kim, S.-T., Kim, H., Jeong, G. and Kang, S.-O. (2001) Infect. Immun. 69, 3939–3946.
[15] Hwang, C.-S., Rhie, G., Oh, J.-H., Huh, W.-K., Yim, H.-S. and Kang, S.-O. (2002) Microbiol. 148, 3705–3713.
[16] Hwang, C.-S., Baek, Y.-U., Yim, H.-S. and Kang, S.-O. (2003) Yeast 20, 929–941.
[17] Fonzi, W.A. and Irwin, M.Y. (1993) Genetics 134, 717–728.
[18] Feng, Q., Summers, E., Guo, B. and Fink, G. (1999) J. Bacteriol. 181, 6339–6346.
[19] Machida, K., Tanaka, T., Fujita, K.-I. and Taniguchi, M. (1998) J. Bacteriol. 180, 4460–4465.
[20] Del Carratore, R., Della Croce, C., Simili, M., Taccini, E., Scavuzzo, M. and Sbrana, S. (2002) Mutat. Res. 513, 183–191.
[21] Jeon, B.W., Kim, K.T., Chang, S.I. and Kim, H.Y. (2002) J. Biochem. 131, 693–699.
[22] Laun, P., Pichova, A., Madeo, F., Fuchs, J., Ellinger, A., Kohlwein, S., Dawes, I., Frohlich, K.U. and Breitenbach, M. (2001) Mol. Microbiol. 39, 1166–1173.
[23] Madeo, F., Frohlich, E., Ligr, M., Grey, M., Sigrist, S.J., Wolf, D.H. and Frohlich, K.-U. (1999) J. Cell Biol. 145, 757–767.
[24] Madeo, F., Frohlich, E. and Frohlich, K.-U. (1997) J. Cell Biol. 139, 729–734.
[25] Carmel-Harel, O. and Storz, G. (2000) Annu. Rev. Microbiol. 54, 439–461.
[26] Spector, D., Labarre, J. and Toledano, M.B. (2001) J. Biol. Chem. 276, 7011–7016.
[27] Greenhalf, W., Stephan, C. and Chaudhuri, B. (1996) FEBS Lett. 380, 169–175.
[28] Kang, J.J., Schaber, M.D., Srinivasula, S.M., Alnemri, E.S., Litwack, G., Hall, D.J. and Bjornsti, M.A. (1999) J. Biol. Chem. 274, 3189–3198.
[29] James, C., Gschmeissner, S., Fraser, A. and Evan, G.I. (1997) Curr. Biol. 7, 246–252.
[30] Kane, D.J., Sarafian, T.A., Anton, R., Hahn, H., Gralla, E.B., Valentine, J.S., Ord, T. and Bredesen, D.E. (1993) Science 262, 1272–1277.
[31] Ghibelli, L., Coppola, S., Rotilio, G., Lafavia, E., Maresca, V. and Ciriolo, M.R. (1995) Biochem. Biophys. Res. Commun. 216, 313–320.
[32] Wiedau-Pazos, M., Trudell, J.R., Altenbach, C., Kane, D.J., Hubbell, W.L. and Bredesen, D.E. (1996) Free Radic. Res. 24, 205–212.
[33] Armstrong, J.S., Steinauer, K.K., Hornung, B., Irish, J.M., Lecane, P., Birrell, G.W., Peehl, D.M. and Knox, S.J. (2002) Cell Death Differ. 9, 252–263.
[34] Finkel, T. and Holbrook, N.J. (2000) Nature 408, 239–247.
[35] Fleury, C., Mignotte, B. and Vayssière, J.-L. (2002) Biochimie 84, 131–141.
[36] Filomeni, G., Rotilio, G. and Ciriolo, M.R. (2002) Biochem. Pharmacol. 64, 1057–1064.
[37] Shi, D.-Y., Deng, Y.-R., Liu, S.-L., Zhang, Y.-D. and Wei, L. (2003) FEBS Lett. 542, 60–64.
[38] Madeo, F., Herker, E., Maldener, C., Wissing, S., Lächelt, S., Herlan, M., Fehr, M., Lauber, K., Sigrist, S.J., Wesselborg, S. and Fröhlich, K.-U. (2002) Mol. Cell 9, 911–917.