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# Asc1p, a Ribosomal Protein, Plays a Pivotal Role in Cellular Adhesion and Virulence in *Candida albicans*

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***Candida albicans,* the common human fungal pathogen, can switch morphology from yeast to pseudohyphal or hyphal form upon various environmental cues. It is well-known that the ability of morphological conversion and adhesive growth renders *C. albicans* virulent. It is noteworthy that every factor involved in the morphogenesis is known to be important for the virulence of this pathogen. To examine a functional relevance of Asc1p, a ribosomal protein, in morphogenesis and virulence, an *asc1* homozygous null mutant was generated. Although a normal morphological transition of the *asc1* deletion strain in liquid media was found, it did not change its morphology on solid media. Moreover, the adhesion activity and hyphal-specific gene expression were defective due to *ASC1* deletion. Finally, it was found that the *asc1* null mutant was avirulent in a mouse model. These results strongly suggested that Asc1p a component of the 40S ribosomal subunit and a signal transducer, plays a pivotal role in cellular adhesion and virulence through regulation of specific gene expression in *C. albicans*.**

***Keywords*:** *C. albicans*, Asc1p, ribosomal protein, virulence, adhesion

*Candida albicans* is an opportunistic fungal pathogen. *Candida* species usually reside as commensal entities and can be detected in approximately 50% of the human population. However, if the immune defenses are compromised, they often become pathogenic (Naglik *et al*., 2003; Netea *et al*., 2008). *C. albicans* expresses several virulence factors that contribute to pathogenesis. These factors include host recognition biomolecules (adhesins), morphogenesis (the reversible transition between unicellular yeast cell and filamentous growth forms), secreted aspartyl proteases, and phospholipases (Calderone and Fonzi, 2001).

A striking feature of *C. albicans* biology is its ability to grow in a variety of morphological forms. These range from unicellular budding yeast to true hyphae with parallel-sided walls (Whiteway and Bachewich, 2007). In between these two extremes, the fungus can exhibit a variety of growth forms that are collectively referred to as pseudohyphae (Crampin *et al*., 2005). The ability to switch between yeast, hyphal, and pseudohyphal morphologies is often considered to be necessary for virulence although formal proof is lacking (Berman and Sudbery, 2002; Sudbery *et al*., 2004).

Asc1p is a component of the 40S ribosomal subunit (McCahill *et al*., 2002) and conserved throughout the eukaryotic kingdom from mammalian *RACK1* to *Saccharomyces cerevisiae CPC2* and *Candida albicans ASC1* (Gerbasi *et al*., 2004; Kleinschmidt *et al*., 2006). In a recent study, *S. cerevisiae ASC1* is not only a small subunit of the ribosome, but is also a key signal trasduction molecule (Nilsson *et al*., 2004; Vomastek *et al*., 2007). Braus and his collegues showed that *ASC1* was required for the expression of *FLO11*, which is a member of the flocculation gene family influencing an adhesion activity of the cell under amino acid starvation conditions in *S. cerevisiae* (Baum *et al*., 2004; Valerius *et al*., 2007). In this study, we was tried to investigate the function of Asc1p influencing a virulence in *C. albicans*.

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## Materials and Methods

### *C. albicans* strains and growth condition

*C. albicans* strains were cultured in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) at 30°C. For confirming deletion strains, heterozygote and homozygote mutants were cultured in Synthetic Complete (SC) medium (0.76% yeast nitrogen without amino acid and 2% dextrose) supplemented with appropriate auxotrophic requirements. To induce hyphal growth of the strains used in this study, cells were grown for overnight in YPD at 30°C and re-cultured into fresh YPD medium with 10% FBS or spider medium (1% nutrient broth, 0.2% $K_2HPO_4$, and 1% mannitol) at 37°C. To observe morphological transition on solid medium, cells cultured overnight in YPD were spotted onto YPD containing 10% FBS or spider medium (1% nutrient broth, 0.2% $K_2HPO_4$, 1.35% agar, and 1% mannitol) plates. The plates were incubated in 37°C for 10 days.

### Plasmids and primers

To generate the expression of *CaASC1* in a yeast strain, pRS315-1150Z vector was digested with *Bam*HI and *Apa*I. The insert (*CaASC1*) was amplified by PCR from BWP17 genomic DNA as a template. Amplified *ASC1* was ligated to the pRS315-1150Z plasmid and the ligation product was used to transform *E. coli* DH5α (NEB, USA). The transformants were selected in LB supplemented with ampicillin (10 μg/ml). The presence of the recombinant DNA was determined by *Bam*HI, *Apa*I, and *Xba*I digestion. The recombinant DNA was used to transform BY4741*Δasc1* cells for complementation.

This transformant was incubated in SC Leu$^-$ media at 30°C until a stationary phase. Expression of *CaASC1* in the yeast strain was confirmed by Northern blot.

**Gene disruption**

The PCR-based gene disruption method was utilized to construct *ASC1* and *ALS3* heterozygous and homozygous mutant strains. Each primer for disruption with *HIS1* and *ARG4* cassette was designed containing 18-24 bp homologous to endogenous *HIS1* and *ARG4* cassette of SC5314 genomic DNA, and a 100 bp of primer each containing *ASC1* or *ALS3* open reading frame. Each auxotrophic marker containing a gene disruption cassette was amplified with 20 pmole forward and reverse primers and 1.5 units of Ex-Taq DNA polymerase (TaKaRa, Japan). Amplification condition were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min 30 sec and a final polymerization performed for 15 min at 72°C. Generally, 200 μl of PCR products were precipitated using ethanol before transformation. The deletion was confirmed by PCR.

***C. albicans* transformation**

BWP17 cultured overnight at 30°C in YPD was reseeded into 30 ml of fresh YPD at $OD_{600}$ of 0.3, and incubated for 4 h at 30°C until $OD_{600}$ was 0.8-0.9. Cells were pelleted by centrifugation at 18,890×g for 5 min. The pellet was washed with 20 ml of distilled water followed by 1 ml of 0.1 M lithium acetate. Cells were resuspended with 300 μl of 1 M LiAc and 50 μl of resuspension was used for each transformation. Cells were pelleted by centrifugation at 18,890×g for 10 sec and the supernatant was discarded. 240 μl of 50% PEG (polyethylene glycol 3350), 35 μl of 1 M LiAc, 5 μl of carrier DNA (boiled for 15 min and cooled directly on ice), and 38 μl of transforming cassette DNA were added to each transformation and mixed by pipetting carefully. These mixtures were incubated overnight at 30°C, heat shocked at 42°C for 30 min, and cooled on ice. Cells were pelleted by centrifugation at 18,890×g for 10 sec and the supernatant was discarded. Cells were resuspended with 100 μl of 1× TE, spread on selective solid media, and incubated at 30°C for 3 days.

**Cell diameters comparison assay and microscopy**

Pre-cultured cells were reseeded at $OD_{600}$ of 1.5 in YPD at 30°C for yeast growth form and in YPD with 10% FBS at 37°C for hyphal growth form. Early log phase cells of BWP17 (WT) and CJK110 (*asc1* null) strains were harvested for taking photos by differential interference phase contrast (DIC) microscopy (Zeiss, Germany). Cell diameter was measured by multi Gauge v.2.3 (FujiFilm, Japan).

**Growth curve**

Overnight cultures of BWP17 (WT), CJK108 (*ASC1* heterozygote), CJK109 (*ASC1* heterozygote), and CJK110 (*asc1* null) strains at 30°C were diluted into fresh 15 ml YPD media at $OD_{600}$ of 0.05. Optical density of each sample was measured every 2 h until a stationary phase with a spectrophotometer (OPTIGEN, USA).

**Northern blotting**

For Northern blot analysis, total RNA was isolated from each culture by a hot-phenol method. 5 μg of total RNA was loaded onto a 1% formaldehyde agarose gel containing morpholinepropanesulfonic acid (MOPS). Following the transfer to nylon membrane (PerkinElmer, USA), the RNA was hybridized to gene-specific nonradioactive DIG-labeled probes. The DNA probes were labeled by random primed incorporation of digoxigenin-labeled 2'-deoxyuridine 5'-triphosphate (DIG-labeled DNA) using the DIG DNA Labeling Kit (Roche, USA) according to the manufacturer's instructions.

**Adhesion and invasion assay**

To observe adhesion activity, overnight cultures of BWP17, CJK108, CJK109, and CJK110 were plated onto solid YPD medium. After 5 days of incubation at 30°C, plates were scanned prior to and after careful washing with streams of water (EPSON, Korea). To observe invasion activity, BWP17 and CJK110 cells were cultured to a stationary phase. Each strain of $2.0\times10^5$ cells were spotted onto solid YPD medium containing 10% FBS. After 10 days of incubation at 37°C, vertical section images of each plate were scanned.

**Mouse survival test**

Cells cultured overnight in YPD at 30°C were washed with 1 ml of distilled PBS and diluted to $OD_{600}$ of 1.0. Blastospores ($2\times10^6$ cells) of wild-type and *asc1* null strains were injected into male 5-week-old Balb/c mice via the lateral tail vein (SAMTAKO, Korea). Survival of the mice was monitored every day for 1 month.

## Results

### *C. albicans* ASC1 is an ortholog gene of *S. cerevisiae ASC1*

To investigate the function of *ASC1* in *C. albicans*, the amino acid sequence of *C. albicans* Asc1p was compared to *S. cerevisiae* Asc1p. The sequences of the two proteins were 67% homologous (Fig. 1A). To verify the orthology of *C. albicans ASC1*, complementation analysis was performed in the *asc1* null strain. The plasmid containing *CaASC1* was used to transform *asc1* null strains. The exogenous expression of *CaASC1* in the null strain was confirmed by Northern blotting (Fig. 1B). It was found that *CaASC1* could compensate for the growth defect of the *asc1* null strain (Fig. 1C). Taken together, these data indicate that *CaASC1* is a *bona fide* gene which encodes a protein that is a functional ortholog of ScAsc1p (Kim, 2010).

### Disruption of the *ASC1* gene in *C. albicans*

An *asc1* null strain was generated from BWP17 using a PCR-based gene disruption method to investigate the *in vivo* functions of this protein. The gene deletion strategy is summarized in Fig. 2. Deletion was confirmed by PCR using genomic DNA as a template with different sets of primers (Fig. 2).

### Cell phenotype was changed by *ASC1* deletion

To check the functions of Asc1p in *C. albicans*, we first observed cell phenotypes of wild-type and null strains. Microscopy analysis revealed an increased cell size of the *asc1* null strain in comparison with the wild-type strain. There was no difference in the shortest diameter between the wild-type and *asc1* null strains. However, the average of longest diameter of *asc1* null cells was 2-fold greater than that of wild-type cells (Fig. 3A). The *asc1* null strain had a longer doubling time than that of the wild-type and other heterozygous mutant cells. This strongly suggests that Asc1p was important for normal growth under physiological conditions (Fig. 3B). To determine whether the *asc1* null strain can change morphology in hyphal-inducing conditions, the wild-type, heterozygous

**(A)**

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CaASC1          MADQEVLVLRGTLEGHNGWVTSLATTPAHPDLLLSGSRDKTLIKWKLTGGEDNQYGIPKK 60
ScASC1          MASNEVLVLRGTLEGHNGWVTSLATSAGQPNLLLSASRDKTLISWKLTG-DDQKFGVPVR 59
RACK1           --MTEQMTLRGTLKGHNGWVTQIATTPQFPDMILSASRDKTIIMWKLTR-DETNYGIPQR 57
                    * :.*****:*******.:***:.  *:::**.*****:* ****  :: ::*:* :

CaASC1          SFKGHSHIVQDVTISADGAYALSASWDRTLRLWDLETGETTQRFVGHKGDVLSVSIAKNL 120
ScASC1          SFKGHSHIVQDCTLTADGAYALSASWDKTLRLWDVATGETYQRFVGHKSDVMSVDIDKKA 119
RACK1           ALRGHSHFVSDVVISSDGQFALSGSWDGTLRLWDLTTGTTTRRFVGHTKDVLSVAFSSDN 117
                :::****:*.* .:::** :***.*** ******: ** * :*****. **:** : ..

CaASC1          RQIVSASRDKTVKVWNTIGECMATLTG--HNDWVSAVRISPS----DQSSTVISASWDKT 174
ScASC1          SMIISGSRDKTIKVWTIKGQCLATLLG--HNDWVSQVRVVPNEKADDDSVTIISAGNDKM 177
RACK1           RQIVSGSRDKTIKLWNTLGVCKYTVQDESHSEWVSCVRFSPN----SSNPIIVSCGWDKL 173
                  *:*.*****:*:*.  * *  *: .  *.:*** **. *.    ...  ::*.. **

CaASC1          VKSWDLADYSVNADFIGHTGYISCITLSPDGSLCASAGKDGVIILWDLNKNKTLYTLEAK 234
ScASC1          VKAWNLNQFQIEADFIGHNSNINTLTASPDGTLIASAGKDGEIMLWNLAAKKAMYTLSAQ 237
RACK1           VKVWNLANCKLKTNHIGHTGYLNTVTVSPDGSLCASGGKDGQAMLWDLNEGKHLYTLDGG 233
                ** *:* : .:::::.***.. :. :* ****:* **.****  :**:*   * :***..

CaASC1          AEVHALAFSPNRYWLAAATTSGIKIFKLQERSLLDELKPEFAVGATAKDP-EAISLAWSA 293
ScASC1          DEVFSLAFSPNRYWLAAATATGIKVFSLDPQYLVDDLRPEFAGYSKAAEP-HAVSLAWSA 296
RACK1           DIINALCFSPNRYWLCAATGPSIKIWDLEGKIIVDELKQEVISTSSKAEPPQCTSLAWSA 293
                  : :*.********.*** ..**::.*: : ::*:*: *.   :.  :* .. ******

CaASC1          DGQNLFAGYTDNVIRVWQVMIPSA 317
ScASC1          DGQTLFAGYTDNVIRVWQVMIAN- 319
RACK1           DGQTLFAGYTDNLVRVWQVTIGTR 317
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**(B)**


**(C)**

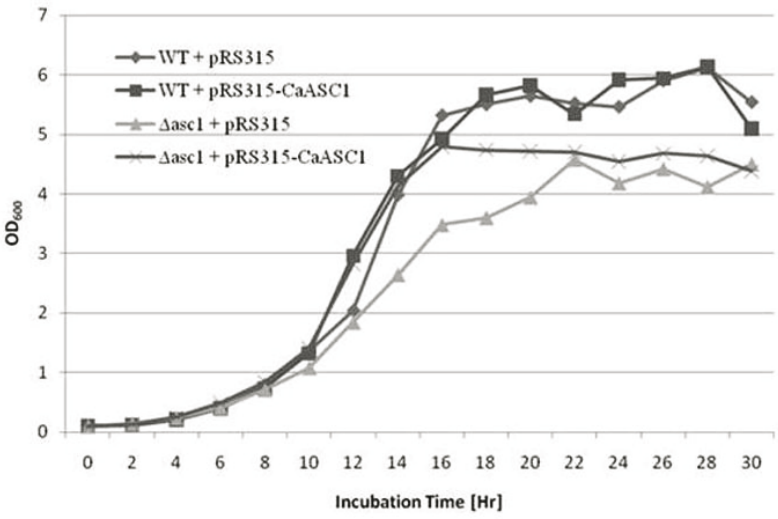


**Fig. 1.** *C. albicans* Asc1p is a functional homologue of *S. cerevisiae* Asc1p. (A) Comparison of predicted amino acid sequences of *C. albicans* Asc1p *(CaASC1)* and *S. cerevisiae* Asc1p *(ScASC1)*. *C. albicans* Asc1p encodes 317 amino acids while *S. cerevisiae* Asc1p encodes 319 amino acids. Identical residues are indicated by asterisks. Similar amino acids between *C. albicans* Asc1p and *S. cerevisiae* Asc1p are indicated by a colon (:). Differences in the amino acid sequence in the two proteins are indicated by a period (.). (B) Plasmids, pRS315 and pRS315-*CaASC1*, were used to transform BY4741 (WT) and BY4741Δ*asc1* (Δ*asc1*) strains. Exogenous expression of *CaASC1* in *S. cerevisiae* was confirmed by Northern blotting with specific probes to *CaASC1* and *ScACT1*. (C) Overnight cultures of each transformant (BY4741+pRS315, BY4741+pRS315-*CaASC1*, BY4741Δ*asc1*+pRS315, BY4741Δ*asc1*+pRS315-*CaASC1*) were transferred to fresh SC-Leu media at $OD_{600}$ of 0.01 at 30°C. The optical density was measured by every 2 h until stationary phase using a spectrophotometer (OPTIGEN).

mutant, and homozygous mutant were cultured in YPD or YPD containing 10% serum after 2 h from reseeding. Wild-type and both mutants not only exhibited normal yeast growth in YPD media, but also exhibited hyphal growth in YPD with 10% serum (Fig. 4A). To determine that morphogenesis in solid media induced hyphal growth, pre-cultured the wild-type, heterozygous mutant, and *asc1* null strain were grown on YPD solid agar plates in the presence or absence 10% FBS or spider medium for 10 days to induce hyphal growth. Wild-type and heterozygous mutant showed hyphal growth as well as typical colony wrinkling. However, the *asc1* null strain showed yeast growth appearing as smooth colonies in both hyphal-inducing solid media (Fig. 4B). Based on these results, it was concluded that Asc1 has contrasting roles in regulating morphogenesis of *C. albicans*. On agar surfaces, Asc1p appears to be a positive regulator of filamentation during growth under hyphal-inducing conditions. In contrast, it does not participate in hyphal growth in liquid cultures.

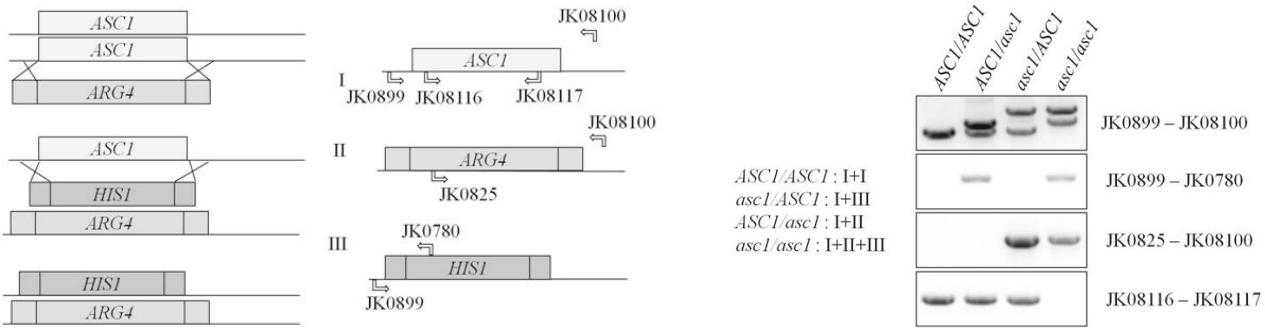


**Fig. 2.** *asc1* null strain was generated by a PCR based gene disruption method. Two *CaASC1* alleles were disrupted with two different deletion cassettes harboring auxotrophic genetic markers, *HIS1* and *ARG4*. Strain generation was confirmed by PCR using 4 sets of specific primers. Heterozygous mutant that was deleted one allele of *ASC1* with *HIS1* cassette was indicated by *ASC1/asc1*. Another heterozygous mutant with an *ARG4* cassette insertion was indicated by *asc1/ASC1*.

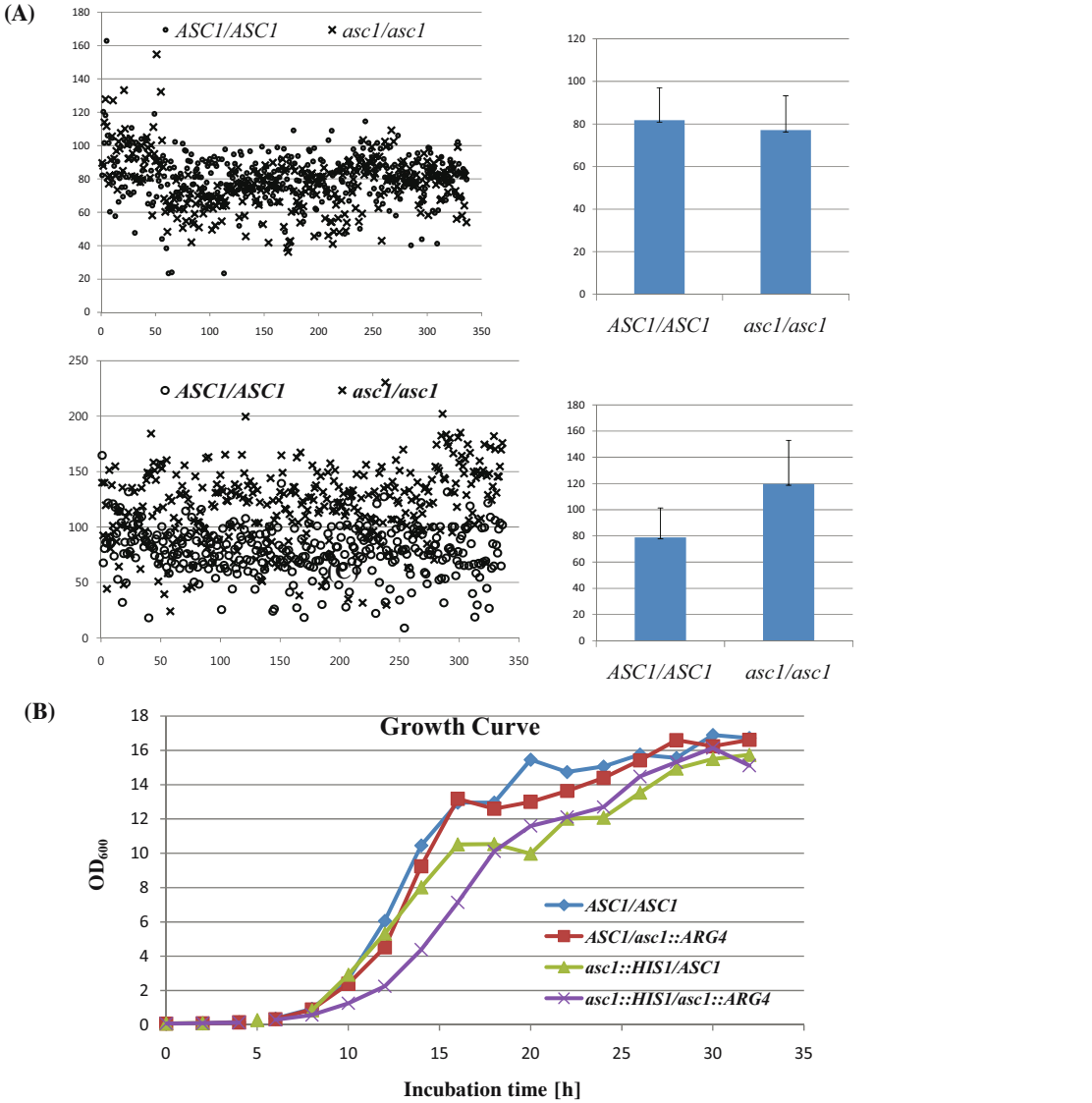


**Fig. 3.** Cell phenotype was changed by *ASC1* deletion. (A) Cell size is regulated by *ASC1.* Overnight cell cultures were transferred to fresh YPD media at $OD_{600}$ of 1.0 at 30°C. Early log phase cells of WT and *asc1* null strain cells were harvested after 2 h. Samples were examined in photos taken by differential interference phase contrast (DIC) microscopy (Zeiss). The number of cells measured was more than 300. Cell diameter was measured by multi Gauge v.2.3 (FujiFilm).

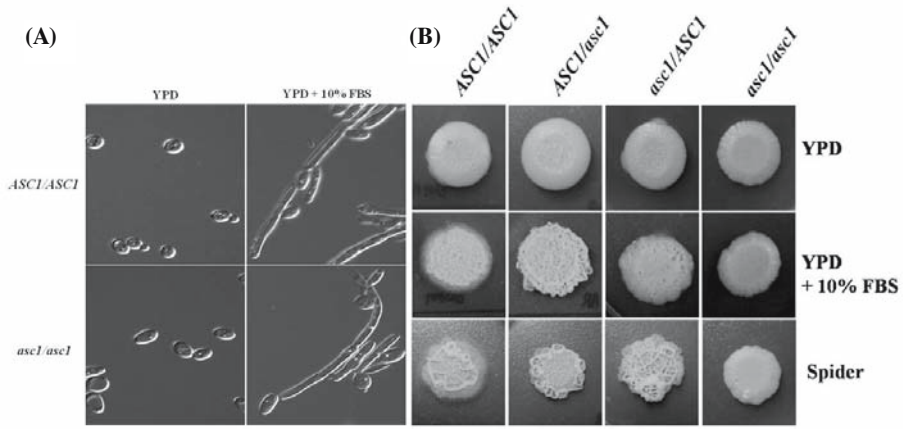


**Fig. 4.** Comparison of the morphogenesis between WT and *asc1* null strain in liquid and solid media. (A) *asc1* null strain has no effect on morphological conversion in liquid hypha induction media. Overnight cultures of WT and *asc1* null strains were used to inoculate YPD or YPD with 10% serum. Samples were examined by photos taken with differential interference phase contrast (DIC) microscopy (Zeiss). Photos were obtained 3 h after reseeding. (B) *asc1* null strain was defective in hyphal growth on agar surfaces. $2.0\times10^5$ cells from overnight cultures of wild-type, heterozygous mutant, and *asc1* null strain were spotted onto spider or YPD plates in the absence or presence of 10% serum. After 10 days of incubation at 37°C, the cultures were photographed with an optical camera (NIKON).

## *asc1* null strain was defective in adhesion growth and invasion

To determine adhesion activities of wild-type and the *asc1* null strain, pre-cultured wild-type, heterozygous mutant, and *asc1* null strain were patched onto YPD plates. After 5 days, the plates were washed carefully with a stream of water as previously described (Li and Palecek, 2003). Loss of adhesive growth was observed in the *asc1* null strain (Fig. 5A). To compare invasive growth of each strain, the wild-type and *asc1* null strain were grown on YPD solid agar media with 10%

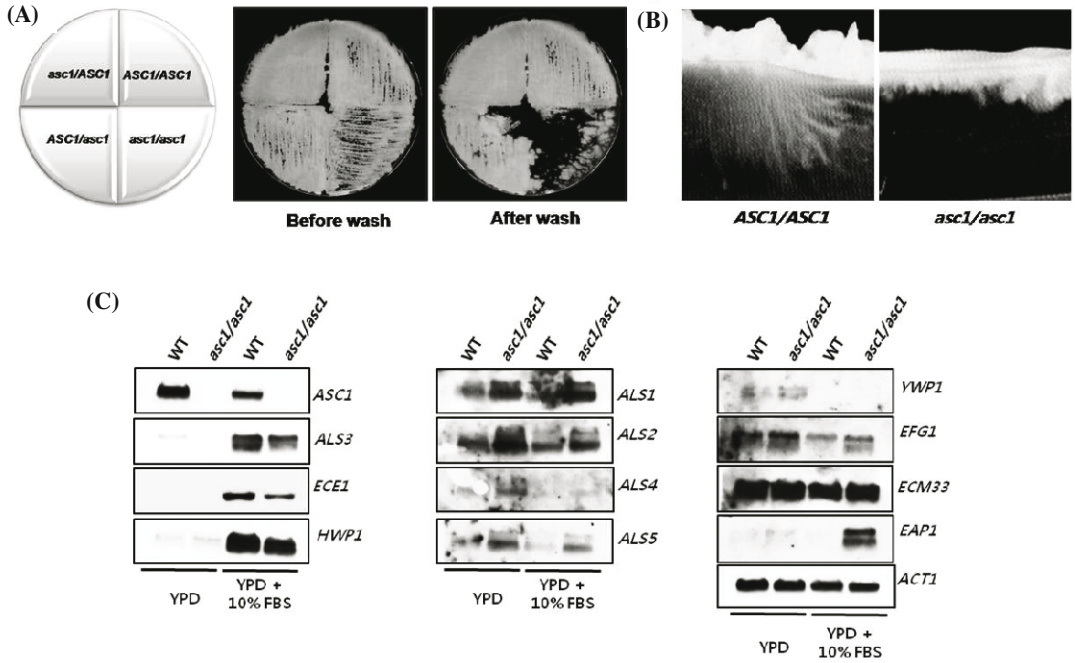


**Fig. 5.** Adhesion and invasion activities of *C. albicans* were regulated by Asc1p. *asc1* null strain was defective in adhesive and invasive growth. Overnight cultures of wild-type, heterozygous mutants, and *asc1* null strain were patched onto solid YPD medium. After 5 days of incubation at 30°C, plates were scanned by an optical camera (SONY) prior to and after washing with a stream of water. (B) Wild-type and *asc1* null strains were cultured to a stationary phase at 30°C, and $2.0\times10^5$ were spotted onto solid YPD medium supplement with 10% serum. After a subsequent incubation for 10 days at 37°C, invasive growth of each strain was investigated by scanning the vertical section. (C) Expression of adhesion genes was down-regulated in an *asc1* null strain. Overnight cultures of wild-type (BWP17) and *asc1* null strain (CJK110) were used to inoculate fresh YPD at $OD_{600}$ of 1.0 in the absence or presence of 10% serum. After 2 h of incubation at 30°C or 37°C, total RNA was precipitated and analyzed on 1% formaldehyde-agarose gels. Northern blotting was performed with DIG-labeled probes against each gene.

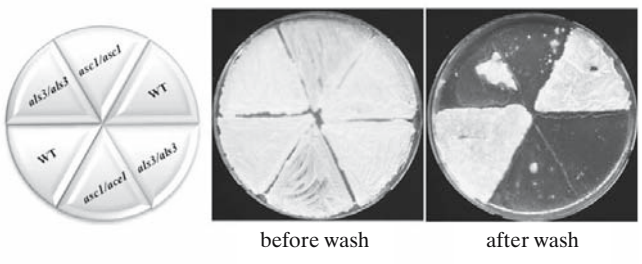


**Fig. 6.** *als3* null strain was defective in adhesive growth. Pre-cultured wild-type, *asc1* null, and *als3* null strains were patched onto solid YPD medium. After 5 days of incubation at 30°C, the plates were scanned prior to and after washing with a stream of water.

FBS for 10 days, and invasive growth of each strain was investigated by scanning vertical sections. The *asc1* null strain was defective in invasive growth into solid agar compared to the wild-type strain (Fig. 5B). The *asc1* null strain was not only defective in invasion activity, but also adhesion activity.

### *ALS3* transcription was down-regulated in the *asc1* null strain

The *asc1* null strain was defective in adhesive and invasive growth. Therefore, it was thought that Asc1p may be involved in a specific set of gene regulation as occurred in yeast. To test the roles of the Asc1p target genes that are related to adhesive and invasive growth, Northern blotting was performed with DIG-labeled probes against 12 genes encoding cell wall proteins and one gene encoding a transcription factor for the expression of hyphae specific genes. Transcription of *ALS3*, *ECE1*, and *HWP1* was decreased via the *ASC1* deletion (Fig. 5C). This observation implied that Als3p, Ece1p, and Hwp1p are key mediators of Asc1p-dependent adhesive and invasive growth.

### *als3* null strain was defective in adhesive growth

To check for the same defective adhesive growth phenotype observed in the *asc1* null strain, *als3* was deleted by a PCR-based gene disruption method. By Northern blotting, the *als3* deletion was confirmed (data not shown). To assess the adhesion activity in the *als3* null strain, overnight cultures of wild-type, *asc1* null, and *als3* null cells were patched onto YPD plates. After 5 days of incubation at 30°C, the plates were carefully washed with a stream of water. The *als3* null strain was washed from the plate as expected (Fig. 6). This result indicates that Als3p appears to be required for adhesive growth on solid media.

### Deletion of *ASC1* rendered *C. albicans* avirulent

In order to establish the role of Asc1p as a virulence factor during host interaction, the virulence of the wild-type and *asc1* null strain was examined in a mouse model of disseminated infection as previously described (Johnston *et al*., 2009). BALB/c mice were inoculated with approximately $2\times10^6$ blastopores of each *C. albicans* strain, and their survival was observed for 30 days. All of the mice inoculated with the wild-type strain died within 16 days except one. Mice infected with the *asc1* null strain were completely asymptomatic (Fig. 7).

## Discussion

*C. albicans* is the most common fungal pathogen of humans, yet progress in understanding its virulence has been difficult. In this study, Asc1p was found to be involved in adhesive, invasive growth and virulence in *C. albicans*. It was also discovered that Asc1p was important for intrinsic regulation of gene expression involved in adherence of *C. albicans*. The null strain of *ASC1* was avirulent in a mouse model. The proposal that Asc1p is a positive regulator of adherence stems from two prior observations. First, Asc1p is required for an adhesion process that depends upon both cell-cell and cell-substrate adherence. Second, numerous Asc1p-dependent genes encode proteins that contribute to cell walls or cell surface structures.

*asc1* null mutant has differential phenotypes in solid and liquid media. There are two kinds of PKAs, Tpk1p, and Tpk2p, influencing the morphogenesis of *C. albicans* in differential environmental conditions. They are required for differential environmental conditions to exert their stimulatory effect. Tpk1p is needed especially to allow hyphae formation on solid inducing media. On the other hand, Tpk2p is needed for the fast hyphae induction that occurs in liquid inducing media or

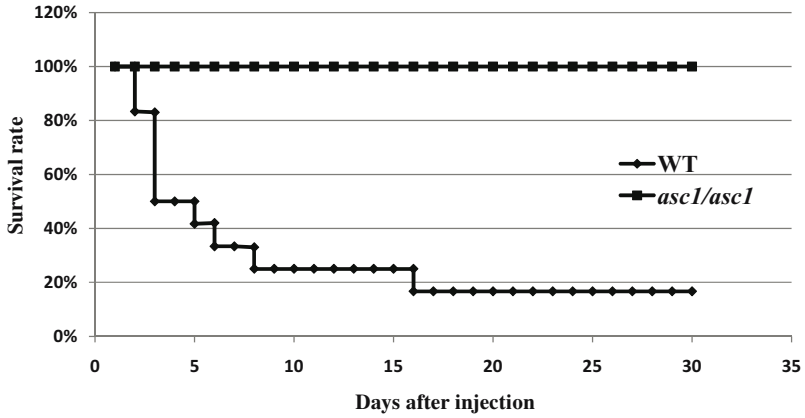


**Fig. 7.** Asc1p is required for *C. albicans* virulence. Wild-type and *asc1* null strains were cultured to stationary phase. These samples were washed with 1 mL of distilled PBS and diluted to $OD_{600}$ of 1.0. Blastospores ($2\times10^6$ cells) of wild-type and *asc1* null strains were injected to 5-week-old male Balb/c mice via the lateral tail vein. Survival of the mice was monitored every day for 1 month.

on solid media at lower temperatures (Bockmuhl *et al*., 2001). Deletion of *asc1* might relate with the signaling pathway of Tpkp isoforms, because the structure of Asc1p is a similar to G-beta subunit. Two activated PKAs by Asc1p might control the morphogenesis of *C. albicans* in differential environmental conditions through the cAMP-dependent or independent signaling pathway.

In *S. cerevisiae*, Asc1p is required for the expression of *FLO11* which is one of the cell surface adhesins because the phosphorylation status of translation initiation factors is regulated by Asc1p (Valerius *et al*., 2007). There is no evidence that Asc1p regulates phophorylation of the translation initiation factors in *C. albicans*, but it is possible because the amino sequences of CaAsc1p and ScAsc1p were 67% homologous and CaAsc1p is a functional ortholog of ScAsc1p. Translation efficiency of transcription factors is influenced by phophorylation of translation initiation factors. Alteration of the expression of transcription factors might result in overall changes in gene expression of a variety of genes including the *ALS3* gene.

In a recent study, protein kinases were found to have crucial roles for cell wall regulation and localization of septin in *C. albicans* (Blankenship *et al*., 2010). Protein kinases and protein kinase-related genes are required specifically for responses to cell wall damage or have a role in cell wall biogenesis. If Asc1p has a similar function as a receptor for activated protein kinase, it might regulate the biogenesis of cell wall proteins and also control expression of adhesion genes in *C. albicans*.

Many transcription factors that have functions in regulating adhesion genes have been well-studied. In particular, Bcr1p is a transcription activator of *ALS3* and *ECE1* (Nobile and Mitchell, 2005; Nobile *et al*., 2006). Therefore, the *asc1* null strain phenotype that shows decreased adhesion activity or transcription of *ALS3* might operate in a Bcr1p-dependent manner. Further study differences in expression patterns of many transcription factors between the wild-type and *asc1* null strain will be examined.

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