Biochemical and Biophysical Research Communications **255,** 116–122 (1999)
Article ID bbrc.1999.0158, available online at http://www.idealibrary.com on IDEAL®

SHC1, a High pH Inducible Gene Required for Growth at Alkaline pH in *Saccharomyces cerevisiae*

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Received January 6, 1999

In this study, we carried out a large-scale transposon tagging screening to identify genes whose expression is regulated by ambient pH. Of 35,000 transformants, two strains carrying the genes whose expression is strictly dependent on pH of growth medium were identified. One of the genes with 20-fold induction by alkali pH was identified as SHC1 gene in the Yeast Genome Directory and its expression was the highest at alkaline pH and moderately induced by osmotic stress. However, the gene was expressed neither at acidic pH nor by other stress conditions. The haploid mutant with truncated *shc1* gene showed growth retardation and an abnormal morphology at alkaline pH. On the other hand, the mutant strain carrying the wild-type *SHC1* gene reverted to the mutant phenotype. To confirm that Shc1p is an alkali-inducible protein, a monoclonal antibody to Shc1p was produced. While a 55-kDa protein band appeared on the Western blot of cells grown at alkaline pH, Shc1p was barely detectable on the blots of cells grown in YPD. Our results indicate that yeast cells have an efficient system adapting to large variations in ambient pH and SHC1 is one of the genes required for the growth at alkaline pH.

Intracellular pH (pHi) in eukaryotic cells is strictly regulated. Eukaryotic cells control cytoplasmic pH at 7.0–7.4 by ion transport mechanisms and a high buffering capacity of the cytosol (1). First of all, most enzymes have an optimum pH for maximum activity. The activity of enzymes taking part in the cellular metabolism is pH-sensitive. In mammalian cells, DNA replication is extremely pH-sensitive (2). The contractile activity of acto-myosin has dramatically influenced by small change in pH (3). Microtubule assembly and disassembly is affected by pH with an increased disassembly at alkaline pH (4). Moreover, pH oscillations seem to be important in controlling the cell cycle and proliferative capacity of cells (5). The regulation of the pHi of phagocytic cells is critical to their function and viability (6). Failure to maintain pH homeostasis results in decreased cellular enzyme activity, cellular migration, and microbial function. Yeasts, like many microbes, encounter large variations in ambient pH in their natural environments. Microorganisms capable of growing over a wide pH range require a versatile and efficient pH homeostatic mechanism protecting intracellular processes against extremes of pH. Budding yeasts grow well at acidic pH as well as alkaline pH in the range of 2.5–8.5, and the kinetics of growth and fermentation are not affected between pH 3.5 and 6.0 because of the tight control of intracellular pH (7). Proton or ion transport proteins in plasma membranes and vacuole membrane might play roles in regulating intracellular pH. However, it has not been described or investigated how the pump proteins are regulated, nor how cells sense extracellular pH, nor what signaling components are involved in pH regulation.

Recently, we have developed a large-scale transposon tagging screen to identify genes expressed during the life cycle of budding yeasts as well as the subcellular localization of the products of many yeast genes. It permitted a systematic identification and characterization of genes expressed at specific cell stages or in various culture conditions (8). Yeast genomic DNA library is mutagenized with a Tn3 mini-transposon containing *lacZ*-coding sequence and the mutagenized DNA is introduced into yeasts. The resulting transformants are selected by assaying β-galactosidase activity on X-gal plate. Since the *lacZ* gene lacks both promoter and ATG initiation codon, β-gal production in yeast depend on the promoter activity of an expressed gene. Inversely, by tracing the β-gal activity, we can identify the genes whose expression is dependent on specific external factors. The approach has applied to an extensive screen for pheromone-regulated genes and identified 45 new pheromone-regulated genes (9).

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0006-291X/99 $30.00

Budding yeast is an ideal model organism to study eukaryotic genome. Basic cellular processes in the yeast are similar in most respects to those of other eukaryotic organisms. Furthermore, recent availability of complete genome sequence of *S. cerevisiae* enables us to identify a certain gene with only partial nucleotide sequences, thus reducing the effort of DNA sequencing. In this report, by combining the transposon tagging gene screening system and the *Saccharomyces* Genome Database (SGD), we have identified yeast genes, whose expression is differentially regulated in response to the pH of growth medium. Of the pH-regulated genes, one with higher expression level in response to alkaline pH was identified as SHC1 and further characterized.

MATERIALS AND METHODS

Yeasts, bacterial strains, and media. The following strains of *Saccharomyces cerevisiae* were used: YC1, *MAT**a** cry^r/MATα CRY ura3-52/ura3-52 leu2-Δ98/leu-2Δ98 HIS3/his3-Δ200 TRP1/trp-Δ1 SHC1/shc1::lacZ;* YC2, *MAT**a** cry^r/MATα CRY ura3-52/ura3-52 leu2-Δ98/leu-2Δ98 HIS3/his3-Δ200 TRP1/trp-Δ1 SCY1/scy1::lacZ;* YC3, *MAT**a** cry^r ura3-52 leu-2Δ98 HIS3 trp-Δ1 shc1::lacZ.* Yeast growth media and standard techniques for the manipulation of yeast have been described by Sherman *et al.* (10). The diploid yeast strain (Y800) were described in the previous study (8). *Escherichia coli* strains were routinely grown on LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, solidified with 2% agar). Kanamycin (Km), chloramphenicol (Cm), streptomycin (Sm), ampicilin (Ap), and spectinomycin (Sp) were used at final concentrations of 40, 30, 100, 100, 20 μg/ml, respectively.

Construction of yeast genomic library with lacZ insertions. We have constructed a yeast genomic library in a vector suitable for transposon mutagenesis (8, 11). Genomic DNA was isolated from a yeast strain (Y800) lacking the 2-μm circle plasmid to eliminate high frequency of the 2- μ plasmid genes. Fragments in 2- to 5-kb range were electroeluted and ligated to the cleaved vector, pHSS6. The ligation mixture was introduced into *E. coli* DH5α. To analyze the library, twenty-four random clones were selected. All except two contained inserts and the average size was 3.5 kb. The final library contained a total of 5×10^4 recombinants, representing 10 genome equivalents and >99.9% of the yeast genome. The yeast genomic library was mutagenized with a mini-Tn3::*lacZ*::*LEU2* using modifications of the procedures of Seifert *et al* (11). Mutagenized DNA with inserts was isolated by a large-scale plasmid preparation and digested with *Not*I restriction enzyme. Mutagenized inserts had been returned to yeast by lithium salt-mediated transformation (12).

β-Galactosidase activity assay. β-gal activity was measured in yeast cells permeabilized with acid-washed glass beads (425–600 μm, Sigma). Cells were washed once in a breaking buffer (50 mM sodium phosphate, 1 mM PMSF, 1 mM EDTA, 5% glycerol, pH 7.4), centrifuged 6 s at 12,000 rpm, and resuspended to an OD_{600} of 100–140 in 200 μl volume. For permeabilization an equal volume of acid-washed glass beads were added, vortexed for 2 min, then incubated on ice for 30 s. The permeabilization procedure was repeated 3–4 times. Then, 800 μl of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol, pH 7.0) was added to the permeabilized cells and reaction was initiated at 28°C by adding 200 μl of substrate solution (4 mg/ml ONPG). Color reaction was terminated by the addition of 100 μl of 2.0 M Na_2CO_3 and incubated time (t in min) was recorded. The cell debris was removed by centrifugation and the OD_{420} of the supernatant (200 μl) was determined with a spectrophotometer. Unit was determined as $(OD_{420} \times 1000)/(OD_{600} \times t)$.

Southern blot analysis. For DNA gel blot analysis, yeast genomic DNA was cleaved with *Eco*RI, separated on an agarose gel, and blotted overnight onto a positively charged nylon membrane (Boehringer Mannheim) according to the directions of the manufacturer. The gel blot was probed for 2 h with a digoxigenin-labeled 3-kb *Bam*HI fragment of the *lacZ* gene. The DNA blots were treated with rabbit anti-digoxigenin antibody, followed by the incubation of goat anti-rabbit IgG antibody conjugated with alkaline phosphatase. The color reaction was initiated by adding bromochloroindolyl phosphate and nitroblue tetrazolium.

DNA sequence analysis. Portions of yeast DNA flanking *lacZ* insertions were subcloned into pXT PCR cloning vector by inverse PCR methods. The genomic DNA fragments digested with *Eco*RI were self-ligated to make the DNA fragment circular. The ligation mixture was amplified with a set of primers which corresponding to each termini of known *lacZ* sequence (5′ end of *lacZ* gene; 5′-CGTTGTAAAACGACGGGATCCCCCT-3′, 3′ end of the truncated *lacZ* gene; 5′-GACGACTCCTGGAGCCCGTCAGTAT-3′). The nucleotide sequence was determined by the dideoxy-chain termination method, using T7 polymerase (U.S. Biochemical Corp.).

Expression of Shc1p under the control of GAL-1 promoter. An expression vector (pYES2) was constructed with *SHC1* gene under the control of a GAL-1 promoter (13). Two oligonucleotides (5′-GAATTCATGTCCATGACTATTTGCTCAAATACTCCT-3′ and 5′-GAATTCTTAAATCAACTTTTTGGCTGCCACAGCTTC-3′) were used in the polymerase chain reaction with yeast genomic DNA as a template. The resulting fragment coding full length *SHC1* ORF with a size of 1.5 kb was digested with *Eco*RI and ligated into pYES2 vector. Transformation of pYES2 vector with *SHC1* gene into the mutant cells was performed by standard procedure (10), and the cells were grown on plates containing synthetic complete (SC) medium lacking uracil. Single colonies from the plates were grown in SC medium lacking uracil and containing 4% raffinose. For induction of Shc1p synthesis, the cells grown the 4% raffinose medium overnight were harvested, resuspended in SC medium lacking uracil and containing 2% galactose, and grown for an addition 16 h. The cells were harvested and washed with phosphate-buffered saline once. The cell lysates were prepared by breaking the cells with a mini-beadbeater (Biospec Products, USA) in a lysing buffer containing 50 mM Tris–HCl (pH 7.4), 5% sucrose (w/v), 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin.

Production of GST-Shc1p fusion protein. A 1.5-kb DNA fragment prepared for pYES2 vector cloning as described above was ligated into *EcoRI*-digested pGEXcs expression vector (14). The plasmid construct was transformed into *E. coli* strain BL21. Production of recombinant protein was induced by the addition of isopropyl-β-thiogalactopyranoside to a final concentration of 1 mM and confirmed by Western blot analysis with a monoclonal anti-GST antibody. The recombinant GST-Shc1p protein was used as an immunogen for the generation of antibodies.

Production of monoclonal antibody against Shc1p. A total volume of 0.3 ml was injected into the intraperitoneal cavity for each female BALB/c mouse (50 μg per mouse, 6–8 weeks old). The first injection was followed by three booster injections at 3- to 4-week intervals. The final injection was carried out without adjuvant. The fusion experiments were carried out as previously described (15).

Western blot analysis. Proteins separated by SDS–PAGE were transferred to nitrocellulose membrane by electroblotting (16). The membranes were incubated for 1 h at room temperature in blocking buffer (5% nonfat dry milk in TBS), and then incubated in culture supernatant containing mAbs. After overnight incubation at 4°C on a rocking platform, the membranes were washed with blocking buffer. The membranes were then reacted with a goat anti-mouse IgG-horseradish peroxide (HRP) (Sigma) at a 1:10,000 dilution in

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                    1                                                  50
SHC1 YEAST 340-484  RAFLLFSAAA KRMHIESVYR TAICY--ECG LGVTRNAPKA VNFLTFAATK
SKT5 YEAST 293-437  EAFVLFQAAA KHGHIESAYR ASHCL--EEG LGTTRDSRKS VNFLKFAASR
CSRF CANAL 396-541  EAFILFQSAA KHGHVESAFR TSFCY--EEG LGTGRDSRKA VEFLKIAASR
Y141 HUMAN 299-434  KAVLYYQLAA SQGHSLAQYR YARCLLRDPA SSWNPERQRA VSLLKQAADS
YAIA SCHPO 661-802  KAFELYSLAA KKGHPLSNYR VAVCL--QTG TGVKPDTSKC VAIYKKAAEM
Consensus           KAFLYYQLAA KQGHPLSQYR YARCLLRDPG TGWNPDRQKC VSLLKQAADS

                    51                                                 100
SHC1 YEAST 340-484  NHPAAMYKLG VYSYHGLMGL PDDILTKMDG YRWLRRATSM ASSFVCGAPF
SKT5 YEAST 293-437  NHPSAMYKLG LYSFYGRMGL PIDVNTKING VKWLSRAAAR ANELTAAAPY
CSRF CANAL 396-541  NHPAAMYKLG VYSFYGRMGL PNDMNTKKMG IKWLTRAALV ATELTAAAPY
Y141 HUMAN 299-434  GLREAQ---- --AFLGVLFT KEPYLDEQRA VKYLWLAANN GDSQS---RY
YAIA SCHPO 661-802  DVVEAMFRIA LIYILNGLLGQ KRNISL---G VQWLERACKS KGPESVRAMY
consensus           NLPEAMYRIG LYAFHGLLGT KEPYLDEQRG VKWLWRACNN GDSQSVRARY

                    101                                                147
SHC1 YEAST 340-484  ELANIYMTGY KDLIISDPDY AMALYEKAAA LGHTESARIL EDARRSG
SKT5 YEAST 293-437  ELAKIYHEGF LDVVIPDEKY AMELYIQAAS LGHVPSATLL AQIYETG
CSRF CANAL 396-541  ELGKLYYNGF EDIVLIDKKY GLELFAQAAA LGHLQSAAIL GHHYEIG
Y141 HUMAN 299-434  HLGICYEKGL --GVQRNLGE ALRCYQQSAA LGNEAAQERL RALFSMG
YAIA SCHPO 661-802  ELAKIYEQPD RYGVSATPER KFELYKQSAV YGYAAAQCKL GECYEHG
consensus           HLAKCYEKGF RYGVQRNPGE AMECYQQSAA LGHEAAQERL RECYEHG
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FIG. 1. Blast search of GenBank with the Shc1p amino acid sequence. The search identified five ORFs containing strong regions of homology along 150 residues to each other with no known structural motifs. CSRF CANAL: chitin synthase regulatory factor in *Candida albican*. Y141 Human: a cDNA clone with no known function in human revealed by random cDNA sequencing (Nagase *et al.,* 1995). YAIA SCHPO: an ORF with an unknown function in *Schizosaccharomyces pombe.*

blocking buffer. For the reaction of HRP, the bound antibodies were detected with a chemiluminescence substrate according to the manufacturer's instruction (ECL; Amersham).

RESULTS AND DISCUSSION

Screening of genes whose expression is regulated by alkali pH. To identify genes whose expression is either induced or repressed by an alkali load, a random *lacZ* insertional mutagenesis screen was used. Yeast genomic library was constructed in a vector suitable for transposon mutagenesis, mutagenized with a derivative of mini-Tn3 transposon with *lacZ,* and introduced into diploid yeasts to generate a collection of yeast strains in which *lacZ* is inserted at random locations in the yeast genome. Each transformant was picked up with toothpicks and examined for β-galactosidase production during vegetative growth. The strains showing positive reaction for β-gal staining contain in-frame fusion with *lacZ* coding sequence. The *lacZ* gene lacks an ATG initiator codon. Thus, expression in yeast is primarily dependent on the inserted yeast gene. The use of diploid strain allows for the isolation of alkali-regulated genes that are essential for vegetative growth.

The yeast transformants with in-frame fusion with *lacZ* insertion were collected, replica-plated to the YPD medium that adjusted to pH 9.5 with NaOH, and assayed for β-gal activity on X-gal plates. As a control, the cells were also replica-plated to normal YPD medium. Of 35,000 transformants, 10 strains carrying the genes whose expression appeared to be induced by high pH were screened at the initial attempt. None of them were repressed in expression level by the alkali treatment. Those clone were selected and tested again for alkali-dependent gene expression. Finally, we selected two strains carrying the genes whose expression are strictly dependent on pH of growth medium. To confirm that the blue color signal of the β-gal activity was due to a unique transposon insertion, a Southern blot analysis was performed. A single DNA band in both of the strains indicated that only one copy of the *lacZ* insert transposed onto yeast chromosomal DNA (data not shown).

Identification of a high pH induced gene, SHC1. To know the identity of the pH-inducible genes, portions of the genes franking *lacZ* gene were cloned by inverse PCR and the partial nucleotide sequences of the genes were subjected to a computer-aided search (8). This revealed that the nucleotides were completely identical to ORF sequences classified in the *Saccharomyces* Genome Database. One locus with 20-fold induction to an alkali load was identified as *SHC1,* ORF YER096W, and the other with 5-fold

induction as *SCY1*, ORF YGL083W. We also determined the nucleotide sequence of the junction between the gene and *lacZ* and confirmed that the *lacZ* gene inserted in-frame into the coding region of the gene at base 519. Thus, the truncated *shc1::lacZ* gene contains one third of full length ORF. Since *SHC1* gene responded more dramatically than *SCY1* gene to an alkali load, we further characterized *SHC1* in depth throughout this study.

SHC1 is located about 500 nucleotides apart from *RAD51* gene and has a 1536 bp open reading frame, encoding a protein with 512 amino acids (56.5 kDa) with no intron. Sequence analysis of Shc1p revealed no known structural motifs. Blast search of GenBank with the Shc1p amino acid sequence identified five ORFs containing regions of homology to each other (Fig. 1). Particularly, *SHC1* shares higher sequence similarity with *S. cerevisiae SKT5/CSD4/CSH4*, a gene with a possible role in protoplast regeneration and killer toxin of *K. lactis* resistance, and in chitin synthesis (17, 18). They share 50% amino acid identity in 307 residues overlap. The Skt5p contains hydrophobic segments and is suggested to be a transmembrane protein. In addition, a calcium-binding domain of 12 amino acids was predicted in the center region of the Skt5p (17). However, Shc1p appeared not to contain any transmembrane domains, nor the consensus sequence of a calcium-binding domain. Other three genes also shows a 150 bp region of strong homology with *SHC1*. First one is the chitin synthase regulatory factor (CSRF) gene encoding a hypothetical protein with 687 amino acids from *Candida albicans*. Others are Human *KIAA0141* (19) and *S. pombe YAIA* gene. These two proteins are hypothetical ORFs revealed by human genome project and *S. pombe* genome project, respectively. It is of interest that sizes of Shc1p and KIAA0141 protein are almost identical, being composed of 512 amino acids and 515 amino acid, respectively.

SHC1 gene expression pattern in response to various stress conditions. We surveyed the expression pattern of *SHC1* as a function of growth pH, with the cells with lacZ insertion. To adjust low pH, YPD medium was acidified with HCl. To adjust high pH, we added a non-metabolizable base, NaOH, into YPD medium (from pH 6.5 to pH 9.5). The expression level began to increase sharply around pH 8.0 and was the maximum at pH 10. However, the *SHC1* did not show any response to acidic pH, even at pH 2.5 (Fig. 2A). We next investigated whether *SHC1* responds specifically to alkaline pH or is also expressed in other stress conditions. The strain with *lacZ* insertion was grown in various stress conditions and cell lysates were assayed for β-gal activity. Among the stresses, alkaline pH was the most effective for the induction of β-gal activity (Fig. 2B). High osmosis also elicited a moderate level of β-gal activity. However, heat shock, UV irradiation, ethanol, starvation, oxidative agents, and others failed to induce. Since hydroxyl ion is regarded as an osmoelectrolyte, it may be explained why *SHC1* gene is expressed not only at alkaline pH but also in osmotic stress.

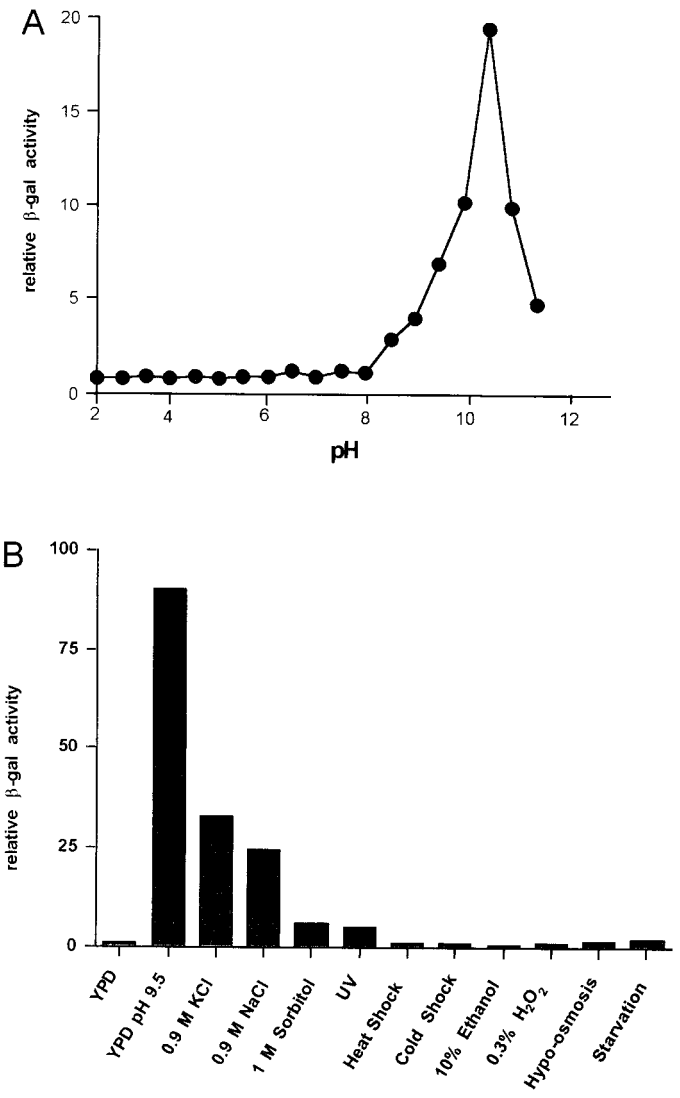

FIG. 2. Responses of SHC1 to various growth conditions. (A) The expression pattern of *SHC1* surveyed as a function of growth pH. The yeast cells with *lacZ* insertion grown in the liquid YPD medium adjusted to different pHs either with NaOH or HCl were permeabilized and β-gal activities were measured at 420 nm using ONPG as a substrate. The expression level began to increase sharply around pH 8.0 and was the maximum at pH 10. However, *SHC1* gene did not show any response to acidic pH, even at pH 2.5. (B) Responses of *SHC1* to various stress conditions. The yeast strain with *lacZ* insertion was grown in various stress conditions and assayed for β-galactosidase activity. In addition to alkali load, osmotic shock also elicited a moderate level of β-gal activity. However, other stress conditions including heat shock, UV irradiation, ethanol, starvation, and oxidative agents failed to induce β-gal activity.

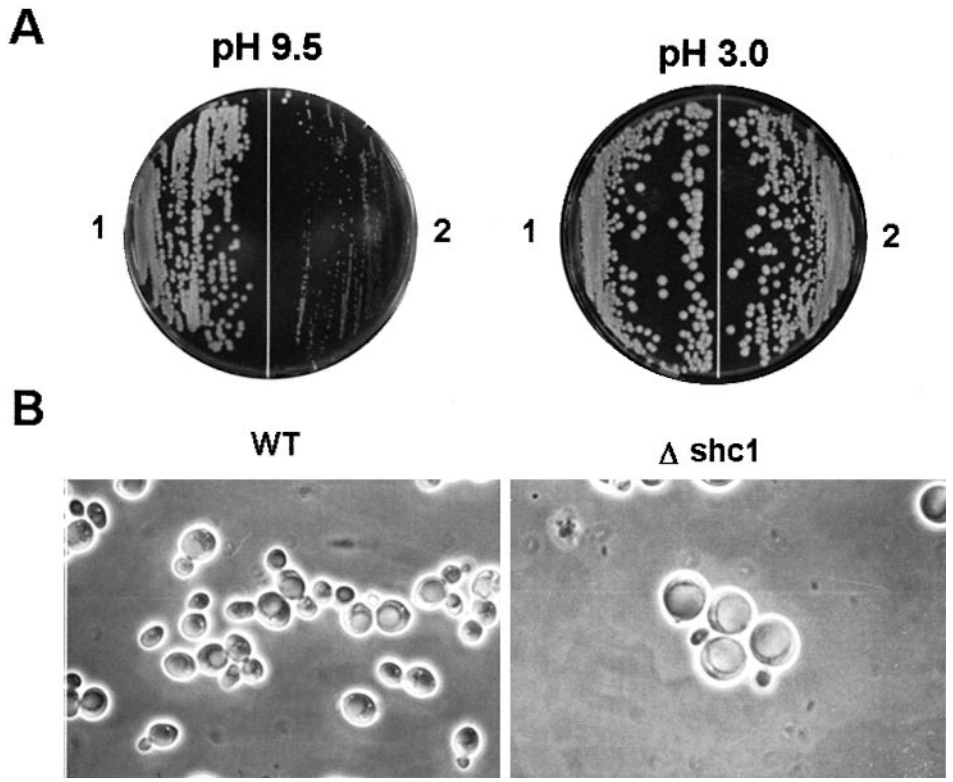

FIG. 3. Growth retardation of *shc1* mutant at alkaline pH. (A) Growth retardation in solid plate. The cells were streaked onto a YPD plate adjusted to pH 7.5 with NaOH, because the wild type do not grown in YDP plate adjusted to higher than pH 8. 1, wild type. 2, *shc1* mutant. (B) Microscopic examination of *shc1* cells after 5 h at pH 9.5. A large numbers of the cell showed an abnormal morphology at alkaline pH.

Growth retardation of shc1 mutant at alkaline pH. The original transformants carrying *lacZ* fusions are heterozygous for the insertion mutations. To determine the phenotypes of haploid cells carrying chromosomal insertion mutation, the diploid transformants were sporulated. To examine the role of *SHC1* in response to alkaline pH, the mutant cells were streaked onto YPD medium adjusted to pH 7.5 with NaOH. Since the growth rate of wild type in YDP plate adjusted to pH 8.0 was also severely retarded, we used YPD plates at pH 7.5. In contrast to wild-type, *shc1::lacZ* cells were retarded for growth in the medium containing NaOH (Fig. 3A). However, the *shc1* cells grow well in acidic medium, even slightly better than wild type. Similar result was observed in liquid YPD culture adjusted to pH 9.5 with NaOH (data not shown). Microscopic examination revealed that a large numbers of mutant cells appeared to be lysed or arrested in growth with an enlarged morphology (Fig. 3B). Since SHC1 was expressed at high osmotic condition to a moderate level, we studied the response of the mutant cells to osmotic solutes. The mutant showed no retardation in cell growth in YPD containing 1 M KCl or 1 M sorbitol (data not shown).

Western blot analysis of Shc1p. To confirm that Shc1p induced by alkaline pH, we produced a monoclonal antibody (mAb) against Shc1p. A DNA fragment coding ORF was amplified by PCR and cloned into a pGEX prokaryotic expression vector. The GST-Shc1p fusion protein was isolated as an inclusion body and injected into mice to generated mAbs. Six hybridomas secreting mAbs that can react with GST-Shc1p not with GST were selected and characterized. Of the 6 mAbs, one recognized a protein band of 55 kDa, quite similar to the expected size of 56.5 kDa, on Western blot. The 55-kDa protein band corresponding to Shc1p was appeared as a prominent band on the blot of cells grown at alkaline pH. In contrast, the band was barely detectable on the blot of cells grown in YPD (Fig. 4). The result indicates that Shc1p is indeed an alkali-inducible protein. Shc1p was also detected in cells without alkali treatment, which had been engineered to produce Shc1p constitutively under the control of GAL-1 promoter (Fig. 4, lane 3).

A possible role of SHC1 in response to alkali load. We examined the extracellular pH of the mutant cells at time intervals after alkali treatment. Wild-type and *shc1* cells were inoculated into YPD medium adjusted to pH 9.5 with NaOH and pH of the YPD medium was measured. Within 3 h wild-type cells lowered pH of the YPD medium to 7.5, whereas mutant cells to pH 9.25 (Fig. 5). The observation suggests that *SHC1* play a

role in the regulation of external pH, particularly in lowering the pH of growth medium.

In yeast, there have been suggested several mechanisms to regulated external pH, particularly lowering pH. Thus, Shc1p does possibly play an important role in one of pH-regulation systems. During yeast alcoholic fermentation, acetic acid is produced as a by-product. In wine and beer fermentation the levels of acetic acid produced may be 1 to 2 g/liter (20). The primary response to a rapid alkali load appears to be the secretion of acetic acid into culture medium and Shc1p could play active role in the process. Second, the plasma membrane H^+-ATPase (PMA1) of the yeast is known to play an essential role in the maintenance of intracellular pH (21). When challenged with glucose, the H^+-ATPase is activated, causing an extensive acidification of the external medium (22). Similar to the dramatic effect of glucose, Shc1p may regulated H^+-ATPase directly or indirectly, thus lowering external pH. Third, in microorganisms such as bacteria, algae, and yeast a large amount of polyphosphate is present up to 10–20% of dry weight under certain conditions (23). One proposed function for the polyphosphate is buffering of intracellular pH (24, 25). It is suggested that the hydrolysis of polyphosphate is a protective mechanism against alkaline stress.

An earlier report suggested that Shc1p is required for chitin synthesis during sporulation (26). While undetectable at vegetative growth, *SHC1* mRNA rises dramatically during sporulation. However, there is no evidence that *SHC1* plays a role in chitin synthesis except the blast search result described above. During vegetative growth and shmoo formation, shc1 disruptants synthesize normal amounts of chitin (26). Instead, SHC1 may be involved in the regulation of external pH, particularly acetate. For instance, potassium acetate is routinely used for sporulation. Acetate in sporulation medium could induce SHC1 gene expression.

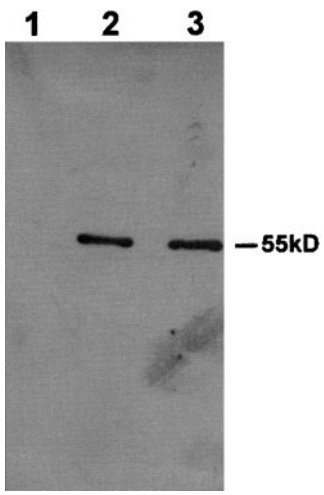

FIG. 4. Western blot analysis of enhanced synthesis of Shc1p in alkali-treated yeasts. A monoclonal antibody against Shc1p was produced from a mouse injected with GST-Shc1p fusion protein as an immunogen. Lane 1: wild-type cell lysates without alkali treatment. Lane 2: wild-type cell lysates treated with alkali. Lane 3: cell lysates from the strain engineered to produce Shc1p constitutively under the control of GAL promoter (see Materials and Methods). While the 55-kDa Shc1p band was barely detectable in cells grown in YPD, the protein was prominent in cells grown at high pH.

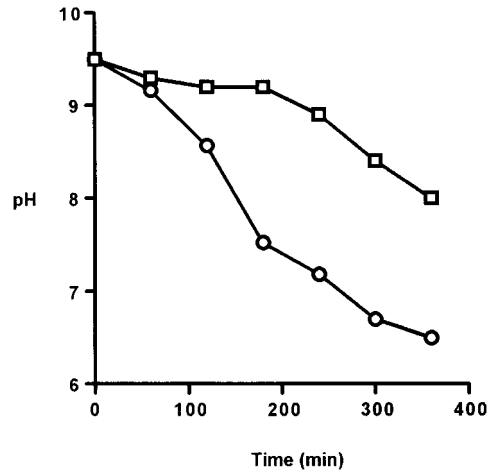

FIG. 5. Recovery pattern of extracellular pH after alkali treatment. Wild-type (□) and *shc1* mutant cells (○) were inoculated into YPD medium adjusted to pH 9.5 and pH of the YPD medium was measured at varying time intervals. Wild-type cells neutralized the alkaline extracellular medium much faster than the mutant strain.

pH regulation in fungi. Although the transmembrane ion pumps and carriers in pH regulation have been intensively studied, little is known about the mechanisms of pH signaling in animal cells. Instead, classical genetic studies have identified many genes that regulate pH homeostasis in fungi (27). In the filamentous fungus, *Aspergilus nidulans*, genetic studies revealed seven genes where mutations in the six *pal* genes can mimic the effects of growth at acidic pH other than the actual ambient pH (27–30). In contrast, mutations in *PacC* mimic the effects of growth at alkaline pH. The *PacC* gene encodes a zinc finger transcriptional regulator which directly regulates expression of gene under pH control (31), and is cleaved into a short version that is active under alkaline condition (32). The products of *pal* genes constitute a signal transduction pathway and trigger the conversion of *PacC* to a functional form. One of the *pal* genes, *palB*, encodes a cysteine protease that does not catalyze proteolytic processing of *PacC* (33). The deduced amino acid sequence from *palA* showed a similarity with Bro1p of *S. cerevisiae*, a novel protein containing an SH3 domain-binding motif (34, 35).

ACKNOWLEDGMENT

This work was supported by Grant KOSEF 981-0505-025-2 from the Korean Science and Engineering Foundation.

REFERENCES

1. Madshus, I. H. (1988) *Biochem. J.* **250,** 1–8.
2. Moolenar, W. H. (1986) *Annu. Rev. Physiol.* **48,** 363–376.
3. Condeelis, J. S., and Taylor, D. L. (1977) *J. Cell Biol.* **74,** 901–927.
4. Regula, C. S., Pfeiffer, J. R., and Berlin, R. D. (1981) *J. Cell Biol.* **89,** 45–53.
5. Aerts, R. J., Durston, A. J., and Moolenaar, W. H. (1985) *Cell* **3,** 653–657.
6. Hackam, D. J., Grinstein, S., and Rotstein, O. D. (1996) *Shock* **5,** 17–21.
7. Matthews, T. M., and Webb, C. (1991) *in* Culture Systems (Tuite, M. F., and Oliver, S. G., Eds.) pp. 249–289, Plenum Press, New York.
8. Burns, N., Grimwade, B., Ross-Macdonal, P. B., Choi, E. Y., Finberg, K., Roeder, G. S., and Snyder, M. (1994) *Genes Dev.* **8,** 1087–1105.
9. Erdman, S., Lin, L., Malczynski, M., and Snyder, M. (1998) *J. Cell Biol.* **140,** 461–483.
10. Sherman, F., Fink, G. R., and Hicks, J. B. (1986) Methods in Yeast Genetics: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
11. Seifert, H. S., Chen, E. Y., So, M., and Heffron, F. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 735–739.
12. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
13. Roy, S. N., Kudryk, B., and Redman, C. M. (1995) *J. Biol. Chem.* **270,** 23761–23767.
14. Parks, T. D., Leuther, K. K., Howard,E. D., Johnston, S. A., and Doughterty, W. G. (1994) *Anal. Biochem.* **216,** 413–417.
15. Choi, E. Y., Park, S. Y., Jang, S. H., Song, M-S., Cho, S-W., and Choi, S. Y. (1995) *J. Neurochem.* **64,** 371–377.
16. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
17. Kawamoto, S., Sasaki, T.,Itahashi, S., Hatsuyama, Y., and Ohno, T. (1993) *Biosci. Biotechnol. Biochem.* **57,** 1391–1393.
18. Trilla, J. A., Cos, T., Duran, A., and Roncero, C. (1997) *Yeast* **13,** 795–807.
19. Nagase, T., Seki, N., Tanaka, A., Ishikawa, K., and Nomura, N. (1995) *DNA Res.* **2,** 167–174.
20. Enrai, T. M. (1995) *Centennial Edition,* 16–18.
21. Serrano, R. (1991) *in* Molecular and Cellular Biology of the Yeast *Saccharomyces* (Broach, J. R., Pringle, J. R., and Jones, E. W., Eds.), pp. 523–585, Cold Spring Harbor Laboratory Press, Plainview, NY.
22. Conway, E. J., and Brady, T. G. (1950) *Biochem. J.* **47,** 360–369.
23. Kulaev, I. S., Szymona, O., and Bobyk, M. A. (1968) *Biochimie* **33,** 419–433.
24. Ludwig, J. R., Oliver, H. S. G., and McLaughlin, C. S. (1977) *Biochem. Biophys. Res. Commun.* **79,** 16–23.
25. Pick, U., Bental, M., Chitlaru, E., and Weiss, M. (1990) *FEBS Lett.* **274,** 15–18.
26. Bulawa, C. E. (1993) *Annu. Rev. Microbiol.* **47,** 505–534.
27. Caddick, M. X., Brownlee, A. G., and Arst, H. N., Jr. (1986) *Mol. Gen. Genet.* **203,** 346–353.
28. Arst, H. N., Jr., Bignell, E., and Tilburn, J. (1994) *Mol. Gen. Genet.* **245,** 787–790.
29. Espeso, E. A., Tilburn, J., Arst, H. A., Jr., and Penalva, M. A. (1993) *EMBO J.* **12,** 3947–3956.
30. Shah, A. J., Tilburn, J., Adlard, M W., and Arst, H. N., Jr. (1991) *FEMS Microbiol. Lett.* **77,** 209–212.
31. Tilburn, J., Sarkar, S., Widdick, D. A., Espeso, E. A., Orejas, M., Mungroo, J., Penalva, M. A., and Arst, H. N., Jr. (1995) *EMBO J.* **14,** 779–790.
32. Orejas, M., Espeso, E. A., Tilburn, J., Sarkar, S., Arst, H. N., Jr., and Penalva, M. A. (1995) *Genes Dev.* **9,** 1622–1632.
33. Denison, S. H., Orejas, M., and Arst, H. N., Jr. (1995) *J. Biol. Chem.* **270,** 28519–28522.
34. Negrete-Urtasun, S., Denison, S. H., and Arst, H. N., Jr. (1997) *J. Bacteriol.* **179,** 1832–1835.
35. Nicklas, M. E., and Yaffe, M. P. (1996) *Mol. Cell. Biol.* **16,** 2585–2593.