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Identification and Characterization of pH-Regulated Genes in *Saccharomyces cerevisiae*

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Yeast, like many other microbes, encounters large variations in ambient pH in their natural environments. Microorganisms capable of growing over a wide pH range require a versatile, efficient pH homeostatic mechanism protecting intracellular processes against extremes of pH. In several organisms, fusions to the bacterial *lacZ* gene have been extremely useful for the identification of genes expressed at different time during the life cycle or under different growth conditions. In this study, using the *lacZ* gene screening system, we surveyed a large number of yeast strains with *lacZ* insertion to identify genes regulated by pH. A yeast genomic library was constructed and inserted with *lacZ* by a shuttle mutagenesis procedure. The yeast transformants were individually picked up with a toothpick, replica-plated, and grown in alkaline pH medium. Among the 35,000 colonies screened, 10 candidate strains were identified initially by the β-gal assay. We finally confirmed two yeast strains carrying the genes whose expression are strictly dependent on pH of growth medium. One of the fusions showing a 10-fold induction in expression level in response to alkali pH was selected and further characterized. The pH-regulated gene was cloned by inverse PCR and a partial sequence of the gene was determined. Identification and characterization of the gene is currently under investigation.

Key words: *Saccharomyces*, transposon, gene screening, pH, β-galactosidase

Cytoplasmic pH in eukaryotic cells is strictly regulated. Eukaryotic cells clamp cytoplasmic pH at 7.0~7.4 by ion transport mechanisms and a high buffering capacity of the cytosol. There are several reasons why intracellular pH must be strictly controlled (14). The activity of a large number of intracellular enzymes taking part in the cellular metabolism is pH-sensitive. An important example is phosphofructokinase, the rate-limiting enzyme of glycolysis. The activity of this enzyme strongly increased with increasing pH over a small pH interval within the physiological range. The contractile activity of purified preparations of actin and myosin has been shown to be dramatically influenced by comparatively small change in pH (5). Also, microtubule assembly and disassembly is affected by pH with an increased disassembly at alkaline pH (19). The efficiency of the conductivity of ion channels is also affected by pH. It appears to be that changes in pH modify the electrical properties of excitable cells. Thus, small pH changes dramatically influence the responsiveness of such cells (16). Also, intercellular communications via gap junctions, which is important in development and in organized functioning of tissues, is highly sensitive to pH changes (24) Moreover, pH oscillations seem to be important in controlling the cell cycle and proliferative capacity of cells. Several line of evidence support that a strictly controlled pHi (intracellular PH) acts as a second messenger in growth control (8, 26).

Yeasts, like many microbes, encounter large variations in ambient pH in their natural environments. Microorganism capable of growing over a wide pH range requires a versatile, efficient pH homeostatic mechanism protecting intracellular processes against extremes of pH and a means of ensuring that activities undertaken beyond the boundaries of pH homeostasis are only attempted at appropriate ambient pH.

A variety of different approaches have been used to identify and characterize genes and their products. In several organisms, fusions to the bacterial *lacZ* gene have been extremely useful in identifying genes expressed at different time during the life cycle (15) or under different growth conditions (4). Recently, a large scale analysis of yeast genes using the *lacZ* fusion method has

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Table 1. Yeast, bacterial strains, and plasmids

Strains or Plasmids	Description	Sources
Yeast *Saccaromyces cerevisiase* Y800	*MATa leu2-Δ98 cry1^R^ / MATα leu2-Δ98 CRY1* *ade2-101 HIS3 / ade2-101 his3-Δ200* *ura3-52 / ura3-52* *lys2-801 / lys2-801* *can1^R^ / CAN1* *trp1-Δ1 / TRP1* *CYH2 / cyh2^R^* *Cir^+^*	(3)
E. coli		
RDP146	F *recA1* (Δ*lac-pro*) *rpsE* (spectinomycin resistance)	(23)
NS2114Sm	F *recA rpsL* (spectinomycin resistant, contains a λ-*cre* lysogen)	(23)
Plasmids		
pHSS6	A genomic library construction vector (23)	
pLB101	containing a gene encoding transposase	(23)
pOX38::mTn3	conjugative F factor derivative which carries mini-Tn3	(23)

been succesfully conducted. It permitted a rapid and systematic identification and characterization of genes expressed during vegetative growth and meiosis as well as the subcellular localization of the products of many yeast genes (3).

S. cerevisiae is an ideal model organism to study eukaryotic genome. Basic cellular processes in yeast are similar in most respects to those of other eukaryotic organisms. In this report, using the *lacZ* fusion method, we describe identification and characterization of yeast genes, which are differentially regulated in response to the pH of growth medium. Of the genes, one showed a high level of expression upon exposure to alkali pH. Thus, we selected the clone and further characterized.

Materials and Methods

Yeast, bacterial strains, and media

Yeast growth media and standard techniques for the manipulation of yeast have been described by Sherman (24). The diploid yeast strain (Y800) used in this study was described in the previous study (3) and listed in Table 1. *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*

General cloning procedures were carried out as described in Sambrook *et al.* (22). A yeast library was constructed as described previously with minor modifications (3). Yeast genomic DNA was isolated (24), digested to an average size of 3 kb with *EcoR*I and *BamH*I. The DNA was size-fractionated in an agarose gel, and the 2~6 kb DNA was electroeluted, ligated into pHSS6 vector predigested with the restriction enzymes (23), and introduced into DH5α.

Shuttle mutagenesis of yeast genomic library

The library pools of *E. coli* were mutagenized separately with a mini-Tn3::*lacZ*::LEU2 using modifications of the procedures of Seifert *et al.* (23). The DNA was isolated from the library pools of *E. coli* and introduced into competent RDP146 (pLB101) cells which contain a plasmid pLB101 (Cm^r) producing a transposase, selecting for Cm and Km. Then, RDP146 (pO×38::m-Tn3) was mated with the transformants of the previous step, selecting for conjugal transfer of the transposon-borne antibiotic resistance gene, the target plasmid, and the transposase producing plasmid by plating the mating mixture on medium containing Km/Ap/Cm. Incubations for bacterial conjugations were carried out for short periods (0.5-1 h) to ensure independent insertions. To allow transposition of mini-Tn3, a culture from the medium containing Km/Ap/Cm was grown at 30°C overnight. The transposition intermediate was resolved by mating the cointegrate into NS2114Sm. An overnight culture of the previous step and an overnight culture of NS2114Sm were diluted separately into fresh LB lacking antibiotics, grown to mid-log phase, mated, and plated onto medium containing km/Ap/Sm. Matings with NS2114Sm were limited to 15 min to avoid more than one transposon insertion. Since NS2114Sm has been known to show unusual high level of endonuclease activity, plasmid DNA was isolated from NS2114Sm and introduced into DH5α. Mutagenized inserts had been returned to yeast by lithium salt-mediated transformation. Mutagenized DNA with inserts was prepared by large-scale plasmid preparation and digested with *Not*I restriction enzyme.

β-galactosidase activity assay

β-galactosidase activity was measured in yeast cells permeabilized with acid-washed glass beads (425~600 microns, Sigma). Cells were washed once in a breaking buff-

er (50 mM sodium phosphate, 1 mM PMSF, 1 mM EDTA, 5% glycerol, pH 7.4), centrifuged 6 seconds 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 seconds. 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 in the permeabilized cells and reaction was initiated at 28℃ 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 $(OD_{420} \times 1,000)/(OD_{600} \times t)$

Southern blot analysis

For DNA gel blot analysis, yeast genomic DNA was cleaved with *EcoR*1, separated on an agarose gel, and blotted overnight onto a positively charged nylon membrane (Boerhinger Mannheim) according to the directions of manufacturer. The gel blot was probed for 2 h with a digoxigenin-labeled 3-kb BamH1 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 BCIP (5-bromo-4-chloro-3-indolylphosphate) and NBT (nitroblue tetrazolium chloride).

Immunofluorescence microscopy

Indirect immunofluorescence was carried out with modifications of published procedure (18). Yeast cells were inoculated into YPD, grown for 12 h at 30℃, and fixed with 3.7% paraformaldehyde for 30 min. After fixation, cells were washed three times with solution A (1.2 M sorbitol, 50 mM KPO_4, pH 7.0), and resuspended in 100 μl of solution A containing 0.1% β-mercaptoethanol and 5 μg/ml of Zymolase 100,000T. After incubation at 37℃ for 1 h, cells were washed twice with PBS (150 mM NaCl, 50 mM $NaPO_4$ at pH 7.4) and once with PBS with 0.1% NP-40. Then, the cells were attached on a 12-mm circular coverslip pretreated with poly-L-lysine, and air-dried. The coverslips were incubated in PBS for 10 min, blocked with 1% bovine serum albumin for 10 min, and treated with rabbit anti-β-galactosidase antibody (Cappel Laboratories) for 1 h. A rat monoclonal antibody against yeast tubulin (YOL1/34, 9) was included as a positive control for the staining procedures. The cells were washed 3 times with PBS containing 0.1% NP-40 and treated for 2 h with goat anti-rabbit IgG antibody conjugated with fluorescein and donkey anti-rat IgG antibody linked to Texas Red (Jackson ImmunoResearch). The cells were mounted and observed with an Olympus epifluorescence microscope.

Inverse PCR

Yeast genomic DNA was isolated from the strains as described above, digested (5 μg) with EcoR1 for 2 h, and phenol-extracted. The DNA fragments were self-ligated overnight to make the DNA fragment circular. The ligation mixture was phenol-extracted again and amplified with a set of primers which corresponding to each termini of known *lacZ* sequence (5' end of *lacZ* gene; CGTTGTAAAACGACGGGATCCCCCT, 3' end of the truncated *lacZ* gene; 5'-GACGACTCCTGGAGCCCGTCAGTAT-3'). The amplified DNA fragments were cloned into a pXT PCR cloning vector.

Results

The procedure for the generation of yeast strains with *lacZ* was divided into two steps, one in bacteria and another in yeast (Fig. 1). A yeast genomic library was constructed and inserted with *lacZ* by bacterial conjugation, utilizing a transposon carrying *E. coli lacZ*. Yeast genomic DNA was isolated and cloned in the pHSS6 vector, which can be used for shuttle mutagenesis procedure. Other cloning vectors, such as pBluescript, pGEM, and pUC vectors, containing a Tn-3 terminal repeat sequence, display immunity to the Tn-3 transposition. The Tn 3 transposition steps was processed by bacterial conjugations. After the two consecutive conjugations, the yeast DNAs with *lacZ* insertions were prepared from *E. coli* and introduced into yeasts by transformation. Since the mutagenized yeast DNAs were linearized with restriction enzyme Not 1 before transformation, they replaced wild type genes by homologous recombination. The yeast transformants were individually picked up with a toothpick, replica-plated, and grown under appropriate conditions to identify yeast genes expressed at the selection conditions.

Construction of yeast genomic library for *lacZ* insertion

We have constructed a yeast genomic library in a vector suitable for transposon mutagenesis. Genomic DNA was isolated from a yeast strain lacking the 2-μ circle plasmid to eliminate high frequency of the 2-μ plasmid genes. Fragments in 2~5 kb range were electroeluted and ligated to the cleaved vector. The ligation mixture was introduced into *E. coli*. To analyze the library, twen-

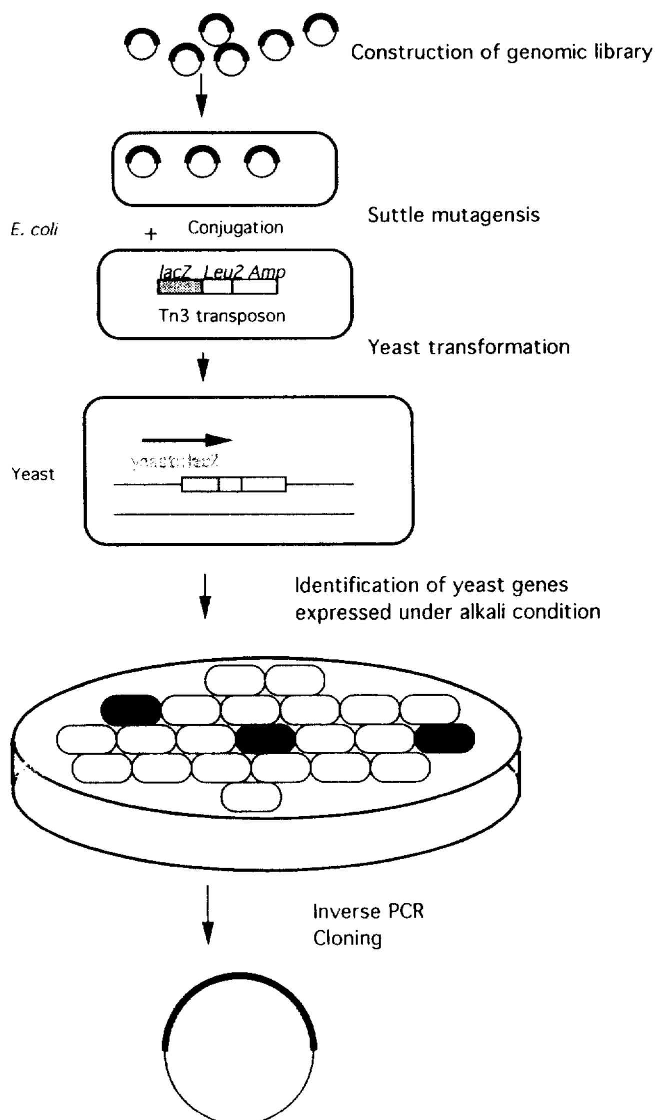

Fig. 1 Overall scheme for generation of yeast::*lacZ* fusions and screening of pH-regulated genes. Details were illustrated in the text (Results).

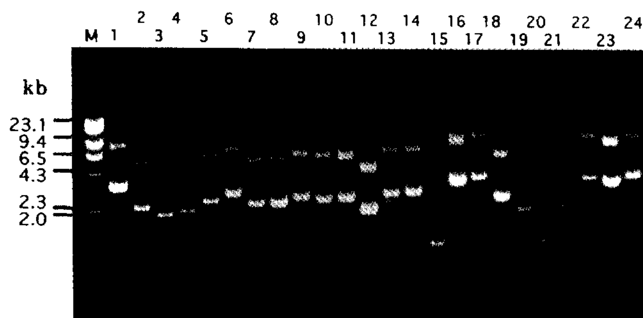

Fig. 2. Construction of a yeast genomic library in the transposition competent pHSS6 vector. After construction, colonies were randomly selected and analyzed for the presence of inserts. Of the 24 colonies tested, two had no inserts. M: molecular weight marker.

ty-four random clones were selected. All except two contained inserts and the average size was 3.5 kb (Fig. 2). The final library contained a total of 5×10^4 recombinants, representing 10 genome equivalents and > 99.9% of the yeast genome.

Transposon mutagenesis

The yeast genomic library was mutagenized with a Tn3 transposon containing *lacZ* coding sequence in order to generate random yeast::*lacZ* fusion genes. The transposon contain at its ends two 38 bp repeats; the *lacZ* coding sequences lie immediately adjacent to one of these repeats and are fused with in frame to an open reading frame (ORF) in the adjacent repeat. The *lacZ* gene lacks promoter sequence and an initiator ATG codon; thus, *lacZ* expression depends on insertion in frame into the coding region of an expressed gene. The transposon also contains the yeast *LEU2* gene for the selection of yeast transformants. The individual pools of recombinant pHSS6 plasmids were mutagenized with the *lacZ-LEU2* transposon. Transposition was allowed to occur for one to two cell divisions, thereby reducing the amplification of plasmids that sustained early transposition events.

Generation and characterization of yeast transformants

DNA from the mutagenized library was digested with *Not*I and transformed into diploid yeast by lithium acetate method (21). Since we used a diploid yeast strain, any of the transformants generated should be heterozygous for the transposon insertion. Individual transformants were patched onto plates and replicas of the patches were grown on Whatman filters. The filters containing yeasts were incubated briefly in chloroform to permeabilize the cells, and then transferred onto plates containing X-gal. Blue patches were identified and quantitatively scored. So far, we have examined about 35,000 transformants and obtained 3,500 blue clones, yielding about 10% frequency. If the entire yeast genome were protein coding sequences and expressed during vegetative growth, the one-sixth (16.7%) of the yeast transformants generated are expected to produce a fusion protein because one-half of the insertions will be in the correct orientation and one-third of these will be in frame. Considering the high copy numbers of rDNA, and that 80% of genes are expressed during vegetative growth, the frequency seemed not to be underestimated.

Identification and characterization of genes regulated by alkali pH

We initially planed to screen yeast genes whose ex-

pression is either induced or repressed by an alkali load as well as an acid load. For unknown reasons, however, we failed to prepare culture plates for acidic pH. The acidic medium was not solidified after plating. To identify genes expressed under alkali condition, the yeast transformants screened as described above were replica-plated to the YPD medium adjusted to pH 9.5 and assayed for β-gal activity on ×-gal plates. As a control, cells were also replica-plated to normal YPD medium. Of 3,500 colonies tested, 10 strains appeared to be positive at the initial attempt. Those clone were selected and tested again. Finally we selected two clones in which β-galactosidase activity increased significantly after an alkali load. We designated for convenience the first gene as *ALk1*, showing 10-fold increase in induction level by alkali treatment, and second one as *ALK2*, 2-fold by the treatment.

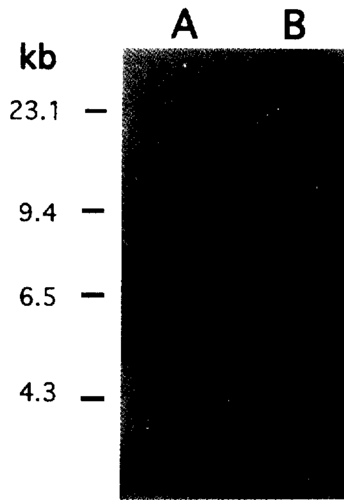

Fig. 3. DNA gel blot analysis of yeast::*lacZ* fusions. DNA from the two candidate strains was cleaved with *Eco*RI and probed with dig-labeled *lacZ* fragment. Single band indicates that only one copy of the *lacZ* insert transposed onto yeast chromosomal DNA. A: *ALK1*, B: *ALK2*.

We investigated whether the yeast strains have more than one transposon insert by Southern blot analysis. When the gel blot was probed with a dig-labeled *lacZ* DNA fragment, a single DNA band was detected in both of the strains. The result indicates that only one copy of the *lacZ* insert transposed onto yeast chromosomal DNA (Fig. 3). Thus, the blue color signal of the β-gal activity is due to a unique transposon insertion.

We further characterized the expression pattern of *ALK1* gene, which showed higher induction level than the *ALk2*, as a function of growth pH (Fig. 4). The expression level began to increase sharply around pH 9.0

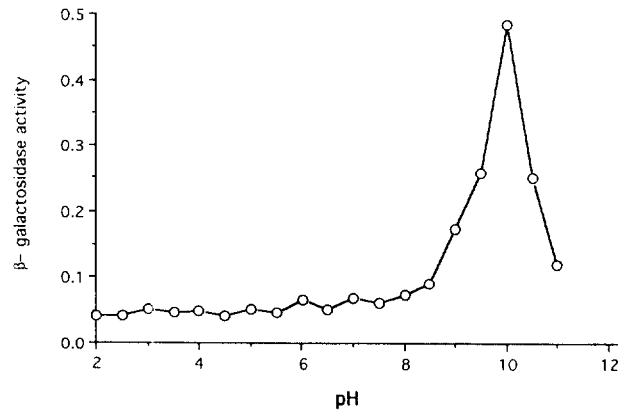

Fig. 4. Expression pattern of the ALK1 gene as a function of pH. The expression level was the highest at pH 10.0. However, it did not respond to acidic pH.

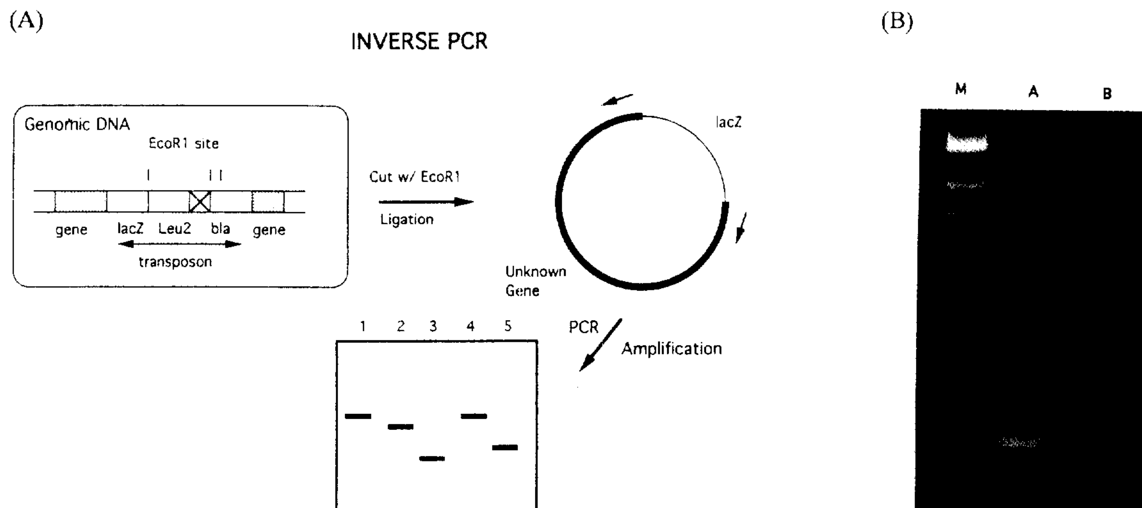

Fig. 5. Inverse PCR amplification of the *ALK1* and *ALK2* genes. (A) General scheme for inverse PCR (B) Amplified DNA fragments. M: molecular weight marker, lane 1: *ALK1*, 2: *ALK2*.

and was the highest at pH 10.0. However, the putative gene did not show any response to acidic pH, even at pH 2.5. To localize ALK1 gene product, the yeast strain was processed for indirect immunofluorescence using rabbit anti-β-gal antibodies. As a positive control, cells were stained with a rat monoclonal antibody that recognizes tubulin (9). The gene product showed a general cytoplasmic staining, suggesting a cytosolic protein (data not shown). However, we could not exclude the possibility that the localization signal of the protein was lost or truncated by the β-gal insert.

Cloning of pH regulated genes

To determine the identity of the genes, the DNA adjacent to *lacZ* was cloned by the inverted PCR method outlined in Fig. 5. Briefly, yeast genomic DNAs from the strains were digested with a restriction enzyme and ligated to make the DNA fragment circular. Then, the gene fragment was amplified with a set of primers which corresponding to each termini of known *lacZ* sequence. The DNA fragments were cloned into pXT PCR cloning vector and is currently being sequenced.

Discussion

A similar approach was applied to prokaryotic cells for the identification of acid-or alkali-inducible genes, including *Salmonella* species. *LacZ* fusions were generated by the Mu*d*-P22 prophage system and specific pH-regulated insertions were identified by transducing LT2 to antibiotics resistance on selective medium (7). From an exhaustive search, 18 pH-controlled *lacZ* operon fusions in *Salmonella typhimurium* have been identified. One of exceptions, the iron-regulated *iroA* locus, was induced by high pH. Whereas, the rest of the pH-regulated genes were expressed under acid condition. In the other prokaryotes extreme alkaliphiles have been extensively studied for the elucidation of pH regulation mechanisms, by which they show an extraordinary capacity for pH homeostasis (11). The alkaliphiles use Na^+/H^+ antiporters in pH regulation and maintain a cytoplasmic pH that is two or more units below the external pH.

In eukaryotic cells, regulation of cytoplasmic pH is mediated by transmembrane acid/base transport pathways and by intracellular buffering systems (21). Three major transport mechanisms have been described and are widely distributed. First, a Na^+/H^+ exchange pathway composed of isoforms that differ in sensitivity to amiloride analogs and that are encoded by the NHE1-4 gene family (6, 28). Second, an anion exchanger, a Na^+-independent $Cl^-/HCO3^-$ exchange mechanism that is inhibited by stilbene disulfonates and that is encoded by AE1-3 genes (1, 10). Finally, a Na^+-dependent $Cl^-/HCO3^-$ exchange mechanism that is inhibited by stilbene disulfonates and is of unknown molecular properties (2, 27). These pathways are reversible, although typically the two Na^+-dependent pathways utilize the inwardly directed Na^+ gradient to produce net acid export, whereas the Na^+-independent exchanger often produces acid import. In addition, specialized pH regulators with more restricted distributions have been characterized (2).

In microorganisms such as yeast a large amount of polyphosphate is present up to 10~20% of dry weight under certain conditions (12). One proposed function for the polyphosphate is buffering of intracellular pH (17). Evidence for buffering is provided by the response of yeast cells to amino acid. Lugwig *et al.* (13), for example, observed extensive tri- and tetrapolyphosphate accumulation after yeast cells were subjected to either an arginine or a lysine bolus. However, acidic or neutral amino acids did not invoke the same response. Therefore, they suggested that low molecular weight polyphosphates contribute to buffering against alkali agents. The alkali-inducible gene, *ALk*1, is induced only by alkali load, and seemed not to be secreted into medium nor located on membranes. Instead, they seemed to be localized as a cytosolic protein. Although more evidences should be accumulated, it could be assumed that *ALK*1 protein could be one of the cytosolic enzymes involved in the polyphosphate metabolism.

In conclusion, we have identified a couple of genes whose expression are regulated by alkali treatment in yeasts using a gene screening system. One of the alkali-inducible genes was cloned and is being almost sequenced. Part of the sequence result has been compared with Stanford *Saccharomyces* Database. The Gene Bank result suggested a new gene, in which only open reading frame was determined by Yeast Genome Project but no known function has been reported yet. The putative yeast gene is being studied to confirm the result.

Acknowledgement

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References

1. **Alper, S.L.,** 1991. The band 3-related anion exchanger (AE) gene family. *Ann. Rev. Physiol.* **53**, 549-564.
2. **Bock, J. and J. Marsh,** 1988. Proton Passage across Cell membrane. Willey, Chichester, UK.
3. **Burns N., B. Grimwade, P.B. Ross-Macdonal, E.Y.**

Choi, K. Finberg, G.S. Roeder, and M. Snyder, 1994. Large-scale analysis of gene expression, protein localization, and gene distruption in *Saccharomyces cerevisiae. Genes & Develop.* **8**, 1087-1105.

4. **Casadaban, M. and S. N. Cohen,** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *In vivo* probe for transcriptional control sequences. *Proc. Natl. Acad. Sci.* **76**, 4530-4533.
5. **Condeelis, J.S. and D.L. Taylor,** 1977. The contractile basis of amoeboid movement. V. The control of gelation, solation, and contraction in extracts from *Dictyostelium discoideum. J. Cell Biol.* **74**, 901-927.
6. **Fliegel, L. and O, Frohlich,** 1993. The Na^+/H^+ exchanger: An update on structure, regulation and cardiac physiology. *Biochem. J.* **296**, 273-285.
7. **Foster, J.W., Y.K. Park, I.S. Bang, K. Karem, H. Betts, H.K. Hall, and E. Shaw,** 1994. Regulatory circuit involved with pH-regulated gene expression in *Salmonella typimurium. Microbiol.* **140**, 341-352.
8. **Gerson, D.F. and A.C. Burton,** 1977. The relations of cycling of intracellular pH to mitosis in the acellular slime mold *Phasarum polycephalum. J. Cell Physiol.* **91**, 297-304.
9. **Kilmartin, J.V., B. Wright, and C. Milsteine,** 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting eat cell line. *J. Cell Biol.* **93**, 576-582.
10. **Kopito, R.R.,** 1990. Molecular biology of the anion exchanger gene *family. Int. Rev. cytol.* **123**, 177-199.
11. **Krulwich, T.A.,** 1995. Alkaliphiles: 'basic" molecular problems of pH tolerance and bioenergetics. *Mol. Microbiol.* **15**, 403-410.
12. **Kulaev, I.S., O, Szymona, and M.A. Bobyk,** 1968. On the synthesis of inorganic polyphosphates in *Neurospora crassa. Biochimie* **33**, 419-433.
13. **Ludwig, J.R., H.S.G. Oliver, and C.S. McLaughlin,** 1977. The effect of amino acids on growth and phosphate metabolism in a prototrophic yeast strain. *Biochm. Biophys. Res. Commun.* **79**, 16-23.
14. **Madshus, I.H.,** 1988. Regulation of internal pH in eukaryotic cells. *Biochem. J.* **250**, 1-8.
15. **O'kane, C.J. and W.J. Gehring,** 1987. Detection in situ of genomic regulatory elements in *Drosophila. Proc. Natl. Acad. Sci.* **84**, 9123-9127.
16. **Moody, W.J. Jr.,** 1982. Intracellular pH: Its Measurement, Regulation, & Utilization in Cellular Functions (Nicciteli, R. & D. W. Deamer, eds.) pp. 427-433, Alan R. Liss, new York.
17. **Pick, U., m. bental, E. Chitlaru, and M. Weiss,** 1990. Polyphosphate hydrolysis-a protective mechanism against alkaline stress? *FEBS Lett.* **274**, 15-18.
18. **Pringle, J.R., A.E. Adams, D.G. Drubin, and B.K. Haaer,** 1991. Immunofluorescence methods for yeast. *Methods enzymol.* **194**, 565-602.
19. **Regula, C.S., J.R. Pfeiffer, and R.D. Berlin,** 1981. Microtubule assembly and disassembly at alkaline pH. *J. Cell Biol.* **89**, 45-53.
20. **Schiestl, R.H. and R.D. Gietz,** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Current genetics.* **16**, 339-346.
21. **Roos, A. and Boron, W.F.** 1981 Intracellular pH. *Physiol. Rev.* **61**, 296-434.
22. **Sambrook, J., E.F. Fritsch, and T. Maniatis,** 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.
23. **Seifert, H.S., E.Y. Chen, M. So, and F. Heffron,** 1986. Shuttle mutagenesis: A method of transposon mutagenesis for Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci.* **83**, 735-739.
24. **Sherman, F., G.R. Fink, and J.B. Hicks,** 1986. Methods in yeast genetics: A laboratory manual. Cold Spring harbor Laboratory, Cold Spring Harbor, New York.
25. **Spray, D.C., J.H. Sternet, A.L. Harris, and M.V.L. Bennet,** 1982. Gap junctional conductance: comparison of sensitivities to pH and Ca++ ions. *Proc. Natl. Acad. Sci.* **79**, 441-445.
26. **Steinhardt, R.A. and M. Morisawa,** 1982. Intracellular pH: Its Measurement, Regulation, & Utilization in Cellular Functions (Nicciteli, R. & D.W. Deamer, eds.) pp. 361-374, Alan R. Liss, new York.
27. **Thomas, R.C.,** 1989. Bicarbonate and pHi response. *Nature* **337**, 601.
28. **Tse, C.-M., S.A. levine, C.H. Yun, S.R. Brant, J. Pouyssegur, B. H. Montrose, and M. Donowitz,** 1993. Functional characteristics of a cloned epithelial Na^+/H^+ exchanger (NHE3): Resistance to amiloride and inhibition by protein kinase C. *Proc. Natl. Acad. Sci. USA* **90**, 9110-9114.