Kor. J. Appl. Microbiol. Biotechnol.
Vol. 19 No. 1, 37-44 (1991)

Elucidation of Function and Isolation of *Trans*-acting Factors Regulating the Basal Level Expression of Eukaryotic Genes

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진핵세포 유전자의 기초대사 발현을 조절하는 *trans* 작용인자의 기능해석과 새로운 인자의 분리

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Abstract — I aimed to isolate *trans*-acting factors involved in the basal expression level of eukaryotic genes. One of the yeast histidine biosynthetic gene, *HIS5* was taken as a model for this study. *HIS5* gene has a substantial basal level in amino acid rich medium and is derepressed if starved for any single amino acid. The derepression is mediated by *cis*-acting DNA sequences 5′-TGACTC-3′ found in 5′ non-transcribed region of the gene and *trans*-acting factors including *GCN4* as positive factor and its negative factor *GCD17*, and *GCN2* as a negative factor of *GCD17*. I first investigated the role of these *trans*-acting factors in *HIS5* basal expression level by using *HIS5-PHO5* fusion in which expression of *PHO5* gene encoding inorganic phosphate-repressible acid phosphatase (APase) is regulated by *HIS5* promoter. Strain with *gcn2* or *gcn4* mutation showed 3 to 4 fold lower APase activity than wild type. The level of APase activity was similar in *gcn2* and *gcn4* mutants. *Trans*-acting factors involved in basal level were identified by isolating 14 mutants showing increased expression of *HIS5-PHO5* fusion from *gcn4* background. All the mutants carry a single nuclear recessive mutation and fall into four complementation groups, designated as *bel1* (basal expression level), *bel2*, *bel3* and *bel4*.

The knowledge of mechanism that regulates transcription of eukaryotic genes has been enriched enormously. Earlier investigations by combined approach of *in vitro* mutagenized gene back into cells identified *cis*-acting regulatory elements called "Promoter". Promoter consists of a proximal element called "TATA box" and distal element spreaded over several hundred base pairs (bp) called "UAS" (upstream activation site) in yeast, and "enhancer" in mammals (1, 2). The TATA box was shown to be a machanical element of promoter, needed to give rise to an mRNA start 60~120 nucleotides downstream in mammalian and most other eukaryotic genes. On the other hand, both UASs (3) and enhancers (4-6) were identified as sites that would activate transcription of adjacent gene. The activities of these *cis*-acting elements are due to transcriptional activator proteins that bind specifically to UASs (7) or enhancers (8) and to the factors that bind TATA box (9). Thus, gene expression is activated or derepressed through interactions of *cis*- and *trans*-acting elements. On the other hand, the repressed or uninduced level of expression is termed as basal expression level. The basal level of expression differs from gene to gene. Since long it has been belived that *cis*-acting elements mainly

Key words: *Trans*-acting factor, basal expression level, repressible acid phosphatase, complementation group
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determines such a basal level expression but not *trans*-acting factors. I initiated the studies on involvement of *trans*-acting factors affecting the basal expression level of eukaryotic gene by taking *HIS5* gene as a model which encodes for histidinol-phosphate aminotransferase (E.C.2.6.1.9) of *Saccharomyces cerevisiae*.

The *HIS5* gene as well as a large number of gens of various amino acid biosynthetic pathways of *S. cerevisiae* are subjected to regulated expression. This regulation is mediated by both *cis*-and *trans*-acting factors. Like other amino acid biosynthetic genes, the 5′ non-transcribed region of *HIS5* contains five copies of consensus sequences, 5′-TGACTC-3′, which has been established to be indispensable elements for derepression for various amino acid biosynthetic genes such as *HIS1*, *HIS3*, *his4* and *TRP5* genes (10). Recently, the importance of these sequences for the full derepression of *HIS5* gene has been suggested using *HIS5-PHO5* gene fusion in which the regulation of *HIS5* gene can be monitored as acticivity of repressible acid phosphatase (APase) of *S. cerevisiae* encoded by *PHO5* gene (11). Multiple *trans*-acting genes are also involved in the regulation of various amino acid biosynthetic genes. Several *GCN* and *GCD* gene products have been identified as positive and negative regulators, respectively (10). Among these, *GCN4* is a most proximal positive regulator and the derepression does not occur in *gcn4* mutant background. However, *HIS5* gene has a substantial basal level expression in the absent of *GCN4*. In this report I isolated mutants showing increased APase activity from *HIS5-PHO5* fusion gene in *gcn4*-deletion mutant background, aiming at identifying *trans*-acting factors that govern the basal expression level of *HIS5*. Results from the genetic characterization of those mutants established four complementation groups.

Materials and Methods

Strains

Principal strains of *S. cerevisiae* used in this study are listed in Table 1. Strains SH2128, SH2136, SH 2142 and SH2146 are transformants of SH1273, SH 1275, SH1996 and SH1089 with plasmid pHRU1, respectively. Mutant strains isolated in this study were listed in Table 5. The presence of *gcn* mutation was judged by the sensitivity resistance aganist 3-aminotriazole (AT) (12). *Escherichia coli* strains JA221 and JM109 were used for the construction and propagation of plasmid DNAs.

Media

For cultivation of yeast cells, YPDA medium (2% glucose, 2% polypeptone, 1% yeast extract and 400 mg/*l* adenine) was used as nutrient medium. To select Ura^+ or Leu^- transformants. Burkholder's synthetic medium (15) supplemented with necessary amino acids, except uracil or leucine, was prepared. For cultivation of *E. coli*, L broth (16) was used with or without sodium salt of ampicillin (50 μg/m*l*). 2% agar was added for solid medium.

Genetic methods

Selection of hybrids subjected to dominance-recessiveness test and complementation analysis was carried out by lawn mating on YPDA and by replica-printing method of the mating patch.

Detection and assay methods for APase

Detection of APase activity in colonies was performed by staining with overlay molten soft agar (1%) containing 0.5 mg α-naphthylphosphate and 5 mg fast blue salt B/m*l* 0.05 M acetate buffer (pH 4.0)(11, 17). Colonies were stained white (no activity), or pink (weak activity) to dark red (strong activity), depending on the APase activity of the cells. To assay for APase activity using intact cells, cells were cultivated in 10 m*l* of YPDA medium at 30℃ for 24 hrs and inoculated into 10 m*l* of YPDA medium as 2% (v/v) and grown for 12 hrs at 30℃. Cells were harvested by centrifugation and suspended in 10 m*l* of 0.01 M acetate buffer (pH 4.0). Assay performed in 1.0 m*l* reaction mixture containing 0.64 mg of ρ-nitrophenyl phosphate and 0.2 m*l* cell suspension at 30℃. One unit of enzyme activity was defined as the amount of enzyme that liberates 1 μmole of ρ-nitrophenol per min at 30℃.

Isolation of mutants

Table 1. *S. cerevisiae* strains

Strain	Genotype	Source
SH518	*MATα, leu2-3,112 lys2*	This work
SH679	*MATα leua-3,112 trp1 pho3-1 pho5-1*	This work
SH1089	*MATα leu2-3,112 trp1 ura3-52 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	This work
SH1091	*MATα ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	This work
SH1249	*MATα gcn2-101 leu2-3,112 pho3-1 pho5-1*	This work
SH1273	*MATα gcn4-103 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	This work
SH1275	*MATα gcn4-103 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	This work
SH1572	*MATα pho2-LEU2 leu2-3-,112 trp1 his3 pho3-1*	This work
SH1996	*MATα gcn2-101 gcd17-501 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	This work
SH2166	*MATα gcn2-101 ura3-52 trp1 his1-29 pho3 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	AT^{s} Segregant of SH2142×SH1091
SH2177	*MATα gcn2-101 gcd17-501 ura3-52 leu2-3,112 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HiS5-PHO5+URA3*]	Segregant of SH2142×SH1091
SH2197	*MATα gcn4-103 ura3-52 trp1 leu2-3,112 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] *HIS5-PHO5+URA3*]	Segregant of SH2128×SH679
SH2207	*MATα pho2-LEU2 ura3-52 trp1 leu2-3,112 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	Segregant of SH1572×SH1089
SH2256	*MATα ura3-52 trp1 leu2-3,112 pho2-LEU2 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*]	Segregant of SH2146+SH2207
SH2257	*MATα gcn4-103 ura3-52 trp1 leu2-3,112 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant showing low APase activity of SH2221×SG21197
SH2260	*MATα gcn2-101 gcn4-103 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant of SH2167×SH2128
SH2313	*MATα bel1-6 gcn4-103 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant of SH2222×SH2257
SH2346	*MATα gcn2-101 gcn4-103 gcd17-501 ura3-52 trp1 leu2-3,112 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant of SH2260×SH2177
SH2347	*MATα gcn2-101 gcd17-501 ura3-52 trp1 leu2-3,112 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	*Segregant of* SH2260×SH2177
SH2381	*MATα gcn4-103 ura3-52 trp1 his1-29 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant of SH2228×SH2257
SH2449	*MATα ura3-52 leu2-3,112 pho3-1 pho5-1* [*HIS4-lacZ, ura3-52*] [*HIS5-PHO5+URA3*]	Segregant of *SH2256*×SH1249

[*HIS4-lacZ, ura3-52*] designates the *HIS4-lacZ* gene fusion contained on a pBR322-derived plasmid (13) integrated between two copies of *ura3-52*. [*HIS5-PHO5+URA3*] designates the *HIS5-PHO5* fusion contained on plasmid pHRU1 integrated between two copies of *ura3-52* locus. The *gcn4-103* allele is a ca. 550 bp deletion of *GCN4* DNA between *Kpn*I site in the GCN4 protein coding sequence (14). *pho2-LEU2* designates the disrupted *pho2* (=*bas2*) gene by *LEU2*.

Cells of parental strain were mutagenized by ethyl methanesulfonate (EMS) (18) and after appropriate dilution cells were plated on YPDA plates and incubated at 30℃. Plates were stained as described above for APase activity and red colonies were screened among pink colonies. Of about 30,000 colonies, I found 14 independent dark or red colonies by this screening method.

Transformation

E. coli was transformed by the method as described by Morrison (19) and that of *S. cerevisiae* was

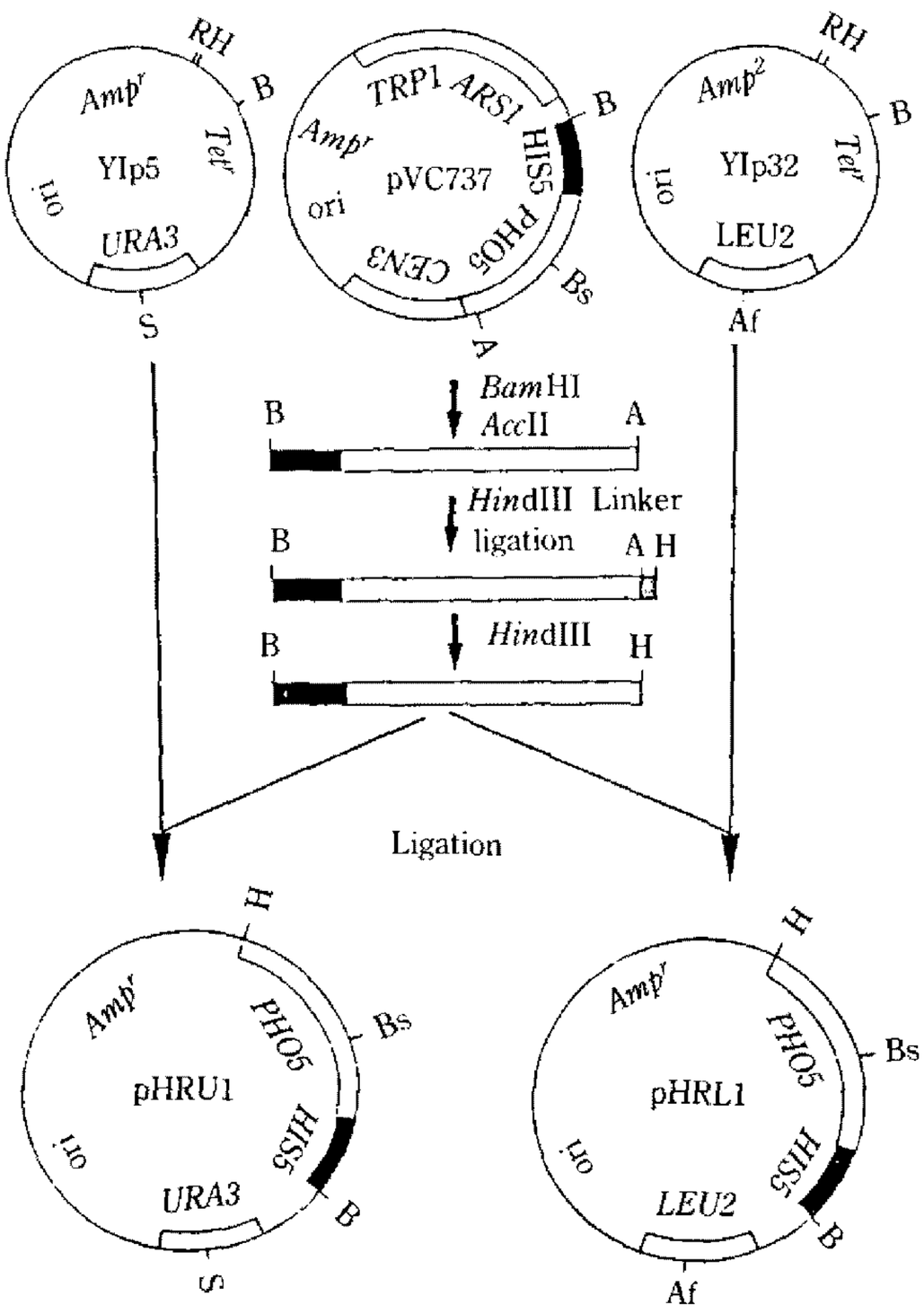

Fig. 1. Construction of integrating plasmids pHRL1 and pHRU1 harboring *HIS5-PHO5* fusion gene.
A single thin line on the circle represents the DNA fragment derived from pBR322. Double thin lines represent the yeast DNA fagment containing *TRP1, ARSI, CEN3, URA3, LEU2* structural part of *PHO5*. A thichk line represents *HIS5* promoter region. The region marked Amp^r and Tet^r are, respectively, the ampicillin and tetracycline resistance gene of pBR322. Ori indicates the region containing the replication origin of plasmid in *E. coli*. The restriction sites for *Acc*II, *Afl*II, *Bam*HI, *Bst*EII, *Eco*RI, *Hin*dIII, *Stu*I are indicated by A, Af, B, Bs, R,H and S, respectively.

by the Li-acetate method of by Ito *et al.* (20).

Modification and analysis of DNAs

Procedure for preparation of plasmid DNA in large scale and for subcloning of DNA fragment are described previously (11, 13, 17). Preparation of yeast chromosomal DNA was performed by the method of Hereford *et al.* (21). Restriction enzyme digestion, ligation of DNAs, isolation of DNA fragments from agarose gels and radiolabeling of DNA fragment were carried out according to established procedures (16).

Plasmid constructions

Construction of integration plasmids with *HIS5-PHO5* fusion is shown in Fig. 1. A plasmid pVC737 was used as source of *HIS5-PHO5* fusion. pVC737 was constructed by first inserting 563 bp *Rsa*I fragment containing *HIS5* promoter region (nucleotide position −597 to −35 relative to the adenine site of the translation initiation codon ATG taken as +1) (22) into *Sma*I site of pUC9 (23). Then, *Bam*HI-*Eco*RI fragment containing the *HIS5* promoter region of the resultant plasmid was subcloned into *Bam*HI-*Eco*RI sit of a promoter-probe vector pVC 728, which was constructed by subcloning *Eco*RI-*Eco*RV fragment from pVC717 (24) into *Eco*RI-*Sma*I site of pUC9 followed Bal31 trimming from *Eco*RI site of resultant plasmid and attachment of *Eco*RI linker (pGGAATTCC; Takara shuzo Co., Japan) (Endpoint after Bal31 deletion was at −34 nucleotide position). A 1.2 kb *Acc*II-*Bam*HI fragment containing *HIS5-PHO5* fusion from pVC737 was fractionated by agarose gel electrophoresis, purified and *Acc*II site was changed to *Hin*dIII by ligation of *Hin*dIII linker d(pCAAGCTTG; Takara shuzo Co., Japan). After linker ligation it was subjected to restriction by *Hin*dIII and finally used for ligation with yeast integration type vectors YIp5 (25) or YIp32 (25) linearized by *Bam*HI and *Hin*dIII. YIp5 and YIp32 contains *URA3* and *LEU2* genes of *S. cerevisiae* as selectable markers, respectively. Frequency of integration of plasmids pHRU1 and pHRL1 at *ura3-52* and *leu2-3,112* was increased by linearizing the plasmids before transformation at the unique site *Stu*I of *URA3* and *Afl*II of *LEU2*, respectivity (26). Plasmid pHK104 was constructed by replacing *Bam*HI-*Bst*EII cassette of pHRU1 by *Bam*HI-*Bst*EII cassette bearing *MFα1* promoter (−963 to 66 as *Eco*RI-*Hin*dIII fragment) (27) fused at +81 of the structural part of *PHO5* gene so as to drive *PHO5* gene by *MFα1* promoter.

Results and Discussion

Analysis of integrated *HIS5-PHO5* fusion

Various strains, SH1273, SH1275, SH1996 and

SH1089 were transformed to Ura$^+$ or Leu$^+$ with either plasmid pHRU1 or pHRL1. Since plasmids were digested within *URA3* or *LEU2* by *Stu*I or *Afl*II, respectively, before transformation, it is highly probable to be integrated at respective locus (26). To confirm this, transformants were crossed with a strain with opposite mating type having *URA3*$^+$ or *LEU2*$^+$ genotype without *HIS5-PHO5* fusion. Resultant hybrids were sporulated and subjected to tetrad analysis. Since no Ura$^-$ or Leu$^-$ segregants appeared in at least 10 tetrads in each hybrid, I concluded that *HIS5-PHO5* fusion is indeed integrated at *ura3-52* or *leu2-3,112* locus of these transformants. Since *HIS5-PHO5* fusion expressed similarly irrespective of the locus where these plasmids were integrated, I used *HIS5-PHO5* fusion integrated at *ura3-52* locus for further study. Single copy integration of pHRU1 at *ura3-52* locus in those transformations was confirmed by Southern hybridization analysis (data not shown).

Effect of *trans*-acting mutations of general control on the basal level expression of *HIS5-PHO5* fusion

Table 2. *gcn2* mutation decreases basal level expression of *HIS5-PHO5* fusion

Spore[a]	Genotype	APase activity mU/m*l*		
		R(YP)[b]	R(SC)[b]	DR[b]
A	+	64.1	40.3	96.8
B	+	40.0	24.5	66.0
C	*gcn2*	17.1	17.1	11.5
D	*gcn2*	14.6	19.6	14.8
Parent I	*gcn2*	15.6	12.1	7.0
Parent II	+	46.0	25.0	84.5

[a] A, B, C and D represent tetrad segregants from hybrids between parental strain I (SH2146) and parental strain II (2166). These strains were cultivated in respective media for 24 hrs and inoculated into the same media as 2% (v/v) and incubated at 30℃ in shaker till absorbancy (660 nm) reached 0.7 to 0.8. APase measurement was carried out as described in Materials and Methods.
[b] R(YP), R(SC) and DR represent APase activity under YPDA, synthetic complete and synthetic complete medium with 1/10 concentration of tryptophan, respectively.

Derepression of many amino acid biosynthetic genes in *S. cerevisiae* is mediated by several *GCN* genes including *GCN2* and *GCN4* as positive factors and *GCD* genes including *GCD17* as a negative factor responding amino acid availability. *GCN4* is a most proximal lpositive regulator which is negatively resgulated by *GCD17. GCD17* is in turn negatively regulated by *GCN2* upon amino acid starvation. This regulation is known as general control (10). The *HIS5* gene has also been shown to be repressed by amino acid starvation (11, 12). I first investigated the role of these *trans*-acting factors in regulation of basal level expression of *HIS5-PHO 5*. I measured APase activities of tetrad segregants from hybrids costructed by crossing *gcn2* with wild type, and *gcn4* with wild type with *HIS5-PHO5* fusion.

Results shown in Table 2 and 3 revealed that the APase was 3 to 4 fold lower in *gcn2* and *gcn4* background in YPDA medium and about 2 fold lower in amino acid complete medium as compared to wild type background. It was also noted that derepression does not occur in *gcn2* and *gcn4* strain as expectedly while 2 to 3 fold derepression was observed in wild type strain under YPDA medium. I also measured APase activity from *HIS5-PHO5* fusion in strains having *gcn2 gcd17, gcn2 gcn4* and *gcn2 gcn4 gcd17* mutation. Strain SH2347 having the genotype of *gcn2 gcn17* showed 4 fold increase of APase level (163 mU/m*l*) relative to wild type strain. These results indicate that *GCN2, GCN4* and

Table 3. *GCN4* contributes to basal level expression of *HIS5-PHO5* fusion

Spore[a]	Genotype	APase activity mU/m*l*		
		R(YP)	R(SC)	DR
A	+	48.0	24.2	73.7
B	*gcn4*	6.7	10.3	4.2
C	+	64.6	22.0	82.0
D	*gcn4*	14.0	17.2	10.0
Parent I	*gcn4*	4.4	8.6	4.6
Parent II	+	46.0	25.3	84.5

[a] A, B, C and D are tetrad segregants from hybrid between parental strain I (SH2136) and parental strain II (SH2146). Other symbols and cultural conditions were the same as described in Table 2.

GCD17 genes are involved in regulation of basal level of *HIS5-PHO5* fusion as well as derepressed level expression as positive and negative factors, respectively, The APase level was more or less similar in *gcn2* (SH2166) (15 mU/m*l*), *gcn2 gcn4* (SH 2260) (19 mU/m*l*) double mutant and *gcn2 gcn4 gcd 17* (SH2346) triple mutant background (36 mU/m*l*) on YPDA medium. This result suggests that *GCN2, GCN4* and *GCD17* play their role in the same regulatory pathway to regulate the basal expression level of *HIS5-PHO5* fusion.

Effect of *gcd17* mutation could be slpecific to *HIS 5-PHO5* fusion

I tested spectificity of *gcd17* mutation on the basal level expression by using *MFα1-PHO5* fusion where *PHO5* gene expression is driven by a promoter of *MFα1* (27) gene encoding mating phermone of *S. cerevisiae*. Wild type (SH1089) and *gcn2 gcd17* (SH1996) strains were transformed with each of plasmid pHRU1 having *HIS5-PHO5* fusion or pHK104 carrying *MFα1-PHO5* fusion and the colonies of these transformants of SH1089 with pHRU1 were pink and those of SH1996 were red while those of transformants of both the strains with pHK 104 were pink by APase staining. This result suggests that the expression of *MFα1-PHO5* fusion is not affected by *gcd17* mutation. I concluded that the effect of *gcd17* mutation could be specific to *HIS5-PHO5* but not general so as to leading to increase in expression of other unrelated genes.

Table 4. *bas2* (=*pho2*) mutation does not affect basal level of *HIS5-PHO5* expression

Spore[a]	Genotype	APase activity mU/m*l*		
		R(YP)	R(SC)	DR
A	*bas2*	16.7	12.5	45.0
B	+	25.2	18.0	32.0
C	+	23.0	20.0	28.0
D	*bas2*	18.6	12.0	45.0
Parent I	*bas2*	14.0	10.0	61.0
Parent II	+	24.0	18.0	28.0

[a] A, B, C and D are tetrad segregants from hybrid between parental strain I (SH2256) and parental strain II (SH2449). Symbols and cultural conditions were the same as described in Table 2.

***bas2* mutation does not affect basal level of *HIS5-PHO5* gene fusion**

The *BAS1* and *BAS2* genes have been identified as positive factors to maintain basal level transcription of *HIS54* in *S. cerevisiae* and shown to bind in the 5′ upstream non-transcribed region of the *HIS4* gene. Genetic mapping and DNA sequence analysis has revealed that the *BAS2* is *PHO2*, a gene previously identified as a positive regulator necessary for derepression of *PHO5* gene (28). The *BAS2* was suggested to be a global regulator of basal level expression of various genes. Therefore, I tested APase level from *HIS5-PHO5* fusion in strain carrying *bas2* (=*pho2*) mutation. APase activity was not significantly different between *bas2* mutant and wild type strain (Table 4). This result suggests that the *BAS2* is not involved in regulation of basal level expression of *HIS5-PHO5* fusion.

Table 5. Results of complementation test

α strain / a strain	SH2257	SH2313	SH2381	SH2456
SH2128	−	−	−	−
SH2211	−	+	−	−
SH2212	−	+	−	−
SH2213	−	+	−	−
SH2220	−	+	−	−
SH2221	−	+	−	−
SH2222	−	+	−	−
SH2224	−	+	−	−
SH2227	−	−	−	−
SH2228	−	−	+	−
SH2229	−	+	−	−
SH2230	−	+	−	−
SH2231	−	−	+	−
SH2233	−	−	−	+
SH2446	−	−	−	−

SH2128 and SH2257 are wild type strains as control. All mutant strains were isolated from SHG2128. SH 2313, SH2381 and SH2455 were segregants showing increased APase activity from hybrids between SH2222 and SH2197 (wild type), respectively. Hybrids were tested for the detection of APase activity on YPDA medium. [+] and [−] represent non-complementation (red) and complementation (white), respectively.

Isolation of mutants showing increased APase activity from *HIS5-PHO5* fusion

As shown in Table 3 the strain carrying *gcn4* mutation fails to derepress the expression of *HIS5-PHO5* fusion. However, substantial basal level of APase activity is observed in the absent of *GCN4*. Colonies of strain SH2128 (=a transformant of strain SH1273 with pHRU1) carrying *gcn4* mutation and *HIS5-PHO5* fusion showed pale pink color when stained for APase detection. This strain was mutagenized by EMS and 14 mutants with red or dark red colonies upon for staining APase were isolated (data not shown).

All the mutations are recessive

All the mutations were tested for dominance-recessiveness with respect to the APase phenotype. This was conducted by staining for APase activity of the hybrids constructed by crossing each mutant with wild type strain SH2257 having *HIS5-PHO5* fusion and opposite mating type. Since colonies of all the hybrids showed pale pink color for APase staining, I concluded that all of these mutations were recessiveness.

Identification of four complementation groups

Among the segregants from hybrids constructed for dominance-recessiveness test, segregants SH 2313, SH2381 and SH2455 with those segregants in all possible pairwise combinations and hybrids were stained for APase activity to determine the number of complementation pattern, all 14 mutants fell into at least four complementation groups. Since SH2227 and SH2446 showed the same complementation pattern, I tentatively assigned these two mutant strains into the same complementation group. However, since complementation test between mutations carried by these two mutants has not been conducted, I cannot eliminate a possibility that they belong to different complementation groups. I designated these complementation groups *BEL* (Basal Expression Level). One complementation group was represented by 9 mutants (SH2211 to SH2224 and SH2229 and SH2230) and was designated as *bel1*, the second contained two members (SH2227 and SH2446) called *bel2* the third contained 2 members (SH2228 and SH2231) called as *bel3* and the fourth contained single member (SH2233) called *bel4* (Table 6).

Table 6. Summary of complementation test

Complementation group	Number of alleles	Name of mutants
BEL1	9	SH2211 (*bel1-1*), SH2212 (*bel1-2*) SH2213 (*bel1-3*), SH2220 (*bel1-4*) SH2221 (*bel1-5*), SH2222 (*bel1-6*) SH2224 (*bel1-7*), SH2229 (*bel1-8*) SH2230 (*bel1-9*)
BEL2	2	SH2227 (*bel2-*), SH2446 (*bel2-2*)
BEL3	2	SH2228 (*bel3-*), SH2231 (*bel3-2*)
BEL4	1	SH2233 (*bel4-*)

요 약

진핵세포 유전자의 기초대사발현의 조절계를 밝히기 위한 일환으로, 효모의 histidine 생합성계 효소의 구조유전자 *HIS5*를 이용하였다. *HIS5* 유전자는 충분한 아미노산 조건하에서는 발현이 억제되어 비교적 높은 기초발현만을 하나, 어떤 아미노산이 결핍되면 탈억제되어 높은 발현량을 보이며 탈억제는 *cis*의 작용인자인 promoter상의 5′-TGACTC-3′ 및 *trans* 작용인자 *GCN4*와 *GCD17 GCN2* 등이 관여한다. *trans* 작용인자들에 의한 *HIS5* 유전자의 발현량의 변화를 간단하게 측정하기 위하여, *HIS5*의 promoter와 repressible acid phosphates(APase)의 구조유전자중 promoter를 제거한 DNA단편을 연결시켜 *HIS5-PHO 5* 융합유전자를 이용하였다. *gcn2* 및 *gcn4* 변이주의 APase 활성은 야생주와 비교하여 3내지 4배 낮았으며, *gcn2*변이주와 *gcn2 gcn4* 이중변이주의 APase 활성은 유사하였다. *HIS5*유전자의 기초대사발현에 관여하는 *trans* 작용인자의 변이주를 분리한 결과, *gcn 4* 배경하에서의 *HIS5-PHO5*의 발현을 증가시키는 14주의 변이주를 분리하였다. 이 변이주들은 단일 핵성의 열성변이주였으며, 네 종류의 complementation group으로 분류되어 *bel1, bel2, bel3* 및 *bel4*로 명명하였다.

Acknowledgement

I especially thank S. Harashima, Department of Fermentation Technology, Osaka Unive., Japan, for advice on construction and mutant analysis of yeast strains.

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(Received January 12, 1991)