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Molecular analysis of *POP2* gene, a gene required for glucose-derepression of gene expression in *Saccharomyces cerevisiae*

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ABSTRACT

We have isolated a new mutant of *Saccharomyces cerevisiae* that exhibits a glucose-derepression resistant (and sucrose-non-fermentor) phenotype. This mutant was obtained by screening for overproduction of α-amylase in a strain containing the mouse α-amylase gene under the control of the *PGK* promoter. The mutation designated *pop2* (*P*GK promoter directed *o*ver *p*roduction). The *pop2* mutant overproduced amylase 5–6 fold and displayed several other pleiotropic defects: (1) resistance to glucose derepression, (2) temperature-sensitive growth, (3) failure of homozygous diploid cells to sporulate and (4) reduced amount of reserve carbohydrates. We mapped *pop2* to chromosome XIV, distal to *lys9* and *SUP28*, indicating that *POP2* is a newly-identified locus. We isolated the *POP2* gene from two yeast strains of different genetic backgrounds, S288C and A364A, and determined their nucleotide sequences. The predicted amino acid sequence of the POP2 protein contains three glutamine-rich region, a proline-rich region and a serine/threonine-rich region, characteristic of many transcription factors. Steady state levels of RNA transcribed from the *PGK-amylase* fusion gene and from endogenous *PGK* gene in stationary-phase *pop2* cells were 5- to 10-fold higher than those observed in wild-type cells, showing that the *pop2* mutation affects transcription of the *PGK* gene transcription.

INTRODUCTION

Glucose regulates the expression of many genes in *Saccharomyces cerevisiae* (see review, ref. 1). One such gene, *SUC2* , encoding invertase, is repressed by glucose and derepressed upon removal of glucose from the medium (2, 3). Many genes that affect the glucose-derepression of *SUC2* gene expression have been reported, such as *SNF1* (*CAT1*, *CCR1*), *SNF2*, *SNF4* (*CAT3*), *SNF5*, *SNF6* and *MSN1* (1). The known gene products of these genes include several transcription factors (SNF2, SNF5 and SNF6) and a protein kinase (SNF1). We previously isolated a mutant, *rgr1*, which is defective in the glucose repression of *SUC2* by fusing the mouse α-amylase gene to the *SUC2* promoter and screening for mutants that overproduce α-amylase in glucose containing medium (4, 5). In order to conduct a similar screen for glucose-derepression mutants we fused the mouse α-amylase gene to the phosphoglycerate kinase gene (*PGK*) and screened for mutants that produce α-amylase in the absence of glucose. The *SUC2* and the *PGK* promoters are regulated by glucose in an opposite manner : *SUC2* is repressed by glucose while *PGK* is activated (6, 7, 8). We reasoned that mutants which affect glucose-derepression may be isolated using the *PGK* promoter.

In this paper, we describe the isolation of a mutant, *pop2*, which is defective in glucose derepression and exhibits a temperature-sensitive growth phenotype. Steady-state levels of *PGK* and *PGK-amylase* gene transcripts in stationary phase cells were significantly higher in *pop2* than in wild-type cells. The *pop2* mutation was mapped to a location on chromosome XIV to which no other mutation has been previously mapped. We isolated the *POP2* DNA and determined the nucleotide sequence. The predicted amino acid sequence contains regions typical of some transcription factors: glutamine-stretches, a proline-rich region and a serine/threonine-rich region. The original *pop2-1* mutation was shown to be a nonsense mutation, and disruption of the *POP2* gene produced the same phenotype as *pop2-1*.

MATERIALS AND METHODS

Strains and genetic methods

The strains of *S.cerevisiae* used in this study are listed in Table 1. All strains were derived from S288C. Crossing, sporulation, and tetrad analysis were carried out by standard genetic methods (9). The permissive and restrictive temperatures were 24°C and 37°C, respectively. *Escherichia coli* strain JM109 (*recA*1 *supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *thi* Δ(*lac-proAB*) F'[*traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*ΔM15] was employed as a host for constructing and propagating plasmids.

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Media

The basic culture medium used for *S.cerevisiae* was YPD medium containing 1% Bacto-yeast extract (Difco), 2% Bacto-peptone and 2% dextrose. Synthetic medium was CSM medium containing 0.67% yeast nitrogen base without amino acids, amino acids as required, and 2% carbohydrate as described in the text. The media were solidified with 2% Bacto-Agar (Difco) for plates. For detection of the activity of secreted mouse α-amylase, YPstarch (YPS) plates containing 1% Bacto-yeast extract, 2% Bacto-peptone, 2% soluble starch (Merck) and 2% Bacto-agar were used. YP sucrose plates containing 1% Bacto-yeast extract, 2% Bacto-peptone, 2% sucrose and 2% Bacto-agar were used to test for the ability to ferment sucrose. Scoring for the fermentation of sucrose was carried out under anaerobic conditions in a Gaspak disposable Anaerobic System (BBL). Luria broth was used for the culture of *E.coli* and was supplemented with ampicillin for selection of transformants as described (10).

Transformations

The transformation of *E.coli* was performed as described (11). The transformation of yeast was performed as described (12).

Construction of an integrative secretion vector of mouse α-amylase and the isolation of single-copy integrant cells

The yeast multicopy secretion vector pREI078 (13), constructed using the promoter of the phosphoglycerate kinase gene (*PGK*), the signal sequence of yeast α factor coded by *MFα1*, the human pro-β-endorphin gene and the terminator of the *MFα1* gene, was digested with *Bam*HI and *Eco*RI. The 1.8 kb DNA fragment bearing the *PGK* promoter and the signal sequence of *MFα1* and the 277 bp DNA fragment bearing the *MFα1* terminator were isolated. These two DNA fragments were ligated with *Eco*RI digested YIp5 whose *Bam*HI site was destroyed by cutting, filling out with Klenow enzyme, and ligating. The resulting plasmid, pSAK028, is a yeast integrative secretion-vector and has a single *Bam*HI site distal to the signal sequence. The pSAK028 plasmid was digested with *Bam*HI and ligated with a mouse α-amylase gene (14) whose own signal sequence was deleted, and the resultant plasmid was designated pSAK031. pSAK031 had a single *Stu*I site at the *URA3* gene and a single *Aat*II site at the pBR322 region. pSAK031 DNA was linearized with *Stu*I prior to transformation. YNN27 cells were transformed with linearized pSAK031. Chromosomal DNA was extracted from the transformants as described (9). The chromosomal DNA was digested with *Stu*I or *Aat*II and electrophoresed in an agarose gel. Single copy integrants were determined by Southern hybridization using ^{32}P-labeled pBR322 DNA as a probe.

Mutagenesis and isolation of mutants

The single-copy integrant of pSAK031 (YNN27/pSAK031/*Stu* I) was treated with 3% ethylmethanesulfonate (EMS) as described (9) and the time of exposure to the mutagen was adjusted to yield a survival rate of 45%. The EMS-treated cells were grown on YPD plates at 24°C for three days. The established colonies were patched on a YPstarch (YPS) plate and incubated overnight at 24°C. The secreted amylase activity was detected by irrigating the colonies on the YPS plates with a solution of 0.2% iodine in 0.2% potassium iodide, as described previously (4). Colonies showing halos larger than that of the original strain were selected. The sizes of the halos of putative mutants were tested three times and the mutants were backcrossed to the wild-type strains at least four times.

Temperature-sensitive growth of strains was determined by their ability to grow on YPD plates at 37°C.

Plasmids

In order to introduce the mouse α-amylase gene in multicopy, a 2-μm-based plasmid pMT56 was used (15).

Preparation of DNA and RNA

Preparation of *E.coli* DNA, Southern and Northern hybridization were performed as described (10). Quantitation of Northern analysis was performed using an image analyzer, BAS2000 (Fuji Film, Japan). Yeast DNAs and total RNAs were prepared as described (9). Pulsed-field gel electrophoresis (contour-clamped homogeneous electric field system) was performed as described (16). The S1 nuclease-mapping experiment was performed as described (5) using a [γ-^{32}P]ATP labelled 330 bp fragment (*Eco*RI-*Sau*I) as a probe.

Enzyme assays

The activity of invertase was determined in whole cells as described (17,18). The activity of secreted amylase in the culture medium was determined as described (19). The activity of isocitrate lyase was determined in crude extracts (20). The concentrations of protein were determined by the dye-binding method (21). The glucose-repressed and -derepressed cells were prepared as described previously (4). In short, log-phase cells (1×10^7 cells/ml) were washed once with YP media containing 5% dextrose ($YPD_{5\%}$) for glucose-repressed cells or $YPD_{0.2\%}$ for glucose-derepressed cells. The cells were resuspended with $YPD_{5\%}$ or $YPD_{0.2\%}$ at cell density of 2×10^7 cells/ml and cultivated at 24°C. Under these conditions, glucose in the derepressed culture media was exhausted after 2−3 hr of incubation. The glucose-derepressed cells were harvested after further 2hr of cultivation.

Estimation of reserve carbohydrates

The content of glycogen in yeast cells was estimated by observing the color of colonies after inverting a plate of cells over iodine vapors. Quantitative measurements of glycogen and trehalose were made according to the method as described (22).

Isolation of the *POP2* gene

The genomic DNA from S288C was partially digested with *Sau*3AI, and the DNA fragments were coned into YEp213. DNA from this library was used to transform A421(carrying *pop2-1*) to leucine prototrophy. The transformants on the selectable plates were transferred to the new plates by replica-plating, and incubated at 37°C for three days. Five transformants were temperature resistant. Plasmid DNAs were recovered from those transformants, all of which contained overlapping DNA fragments. The overlapping DNA fragment was subcloned in YEp213 or in YCp50 plasmid and named YEpPOP2S and YCpPOP2S, respectively.

Sequence analysis

Restriction fragments were cloned into pUC18 and pUC19. Nucleotide sequences were determined (23) with a 7-deaza-Sequenase kit (United States Biochemicals) and [α-^{35}S]dCTP (Dupont, NEN research products). All of the DNA sequences were determined on both strands.

Disruption of *POP2* gene

Alleles *pop2-2::URA3*, *pop2-Δ3::LEU2*, *pop2-Δ4::URA3* and *pop2-Δ5::LEU2* were constructed by one-step gene replacement (24). The 4 kb *Bam*HI fragment carrying *POP2* DNA from YEpPOP2S plasmid was subcloned into pUC19 (pTAK101). The *pop2-2::URA3* was constructed by inserting the 1,565 bp *URA3* fragment with flush end at *Hpa*I site (nucleotide position of 234 from ATG) of pTAK101 and designated pTAK006. The *pop2-Δ3::LEU2* was constructed by deleting the entire coding region (−228 to 2,200) and inserting the 3 kb *LEU2* fragment (pTAK004). The *pop2-Δ4::URA3* was constructed by deleting *Hpa*I-*Nsc*I region (234 to 1,228; approximately 76% of the coding region) and inserting the *URA3* fragment (pTAK014). The *pop2-Δ5::LEU2* was constructed by deleting *Cla*I-*Hin*dIII region (838 to 2,200) and inserting the *LEU2* DNA (pTAK005). DNA fragments carrying the *POP2* disruption (see Fig. 3) were digested by *Bam*HI and used to transform the diploid strain D17 to uracil or leucine prototropy. Transformants in which one copy of the *POP2* gene was disrupted were identified by Southern blot analysis of genomic DNA. These diploids were sporulated and subjected to tetrad analysis to recover haploid segregants carrying the disruption.

RESULTS

Isolation of mutants

Mutants which showed an amylase-overproduction phenotype were selected as described in MATERIALS AND METHODS. Each mutant which originated from YNN27/pSAK031/*Stu*I was crossed to A192/pSAK031/*Stu*I which has an S288C genetic background in order to determine whether the oversecretion phenotype segregated 2:2, as would be expected for a mutation in a single gene. Only recessive mutants which exhibited such 2:2 segregation were characterized further. Among 8,000 colonies screened, only one mutation (*pop2*) was temperature sensitive for growth and exhibited a sucrose-non-fermentor (*snf*) phenotype (see below).

Table 1. List of yeast strains

Strain	Genotype	Source
YNN27	*MATα trp1 ura3 SUC2*	N. Gunge
YNN27/pSAK031/*Stu* I	*MATα trp1 ura3*::pSAK031(*URA3*)[a] *SUC2*	This work
A192	*MATa aro7 can1 leu2 met14 ura3 SUC2*	A. Sakai
A192/pSAK031/*Stu* I	*MATa aro7 can1 leu2 met14 ura3*::pSAK031(*URA3*) *SUC2*	This work
A441	*MATα can1 met14 ura3*::pSAK031(*URA3*) *SUC2*	This work
A448	*MATα aro7 can1 trp1 ura3 SUC2*	This work
A388	*MATa aro7 can1 ura3*::pSAK031(*URA3*) *SUC2 pop1-1*	This work
A391	*MATα aro7 can1 leu2 trp1 ura3*::pSAK031(*URA3*) *SUC2 pop1-1*	This work
A407	*MATα can1 trp1 ura3 SUC2 pop1-1*	This work
A409	*MATa aro7 can1 leu2 trp1 ura3 SUC2 pop1-1*	This work
A417	*MATα can1 ura3*::pSAK031(*URA3*) *SUC2 pop2-1*	This work
A422	*MATa aro7 can1 leu2 trp1 ura3*::pSAK031(*URA3*) *SUC2 pop2-1*	This work
A428	*MATα can1 leu2 met14 trp1 ura3 SUC2 pop2-1*	This work
A429	*MATa can1 leu2 trp1 ura3 SUC2 pop2-1*	This work
MCY1529	*MATa ssn6-Δ5::URA3 lys2-801 ura3-52 SUC2*	M. Carlson
D17	*MATa/MATα ade8/ADE8 aro7/aro7 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3*	This work
YAH89	*MATa leu2-1 lys9 pet8 pho2*	B. Ono
YAH1133	*MATa leu2-1 met4 rad1 ura2*	B. Ono
ST73-10D	*MATa ψ+SUP28$_{UGA}$ his4-166^{u} leu2-2^{u} met8-1^{a} ura4-10^{o}*	B. Ono
ST74-13A	*MATa ψ+ his4-166^{u} ilv1-2^{o} leu2-2^{u} lys9 pet8 ura3 ura4-10^{o}*	B. Ono

[a]A single copy integration of pSAK031(*URA3*) linearized with *Stu* I at *ura3* gene.

cis- and *trans*-acting test

To determine whether the mutations were *cis*-acting or *trans*-acting, the *pop2-1* strain was crossed with A192, a strain which contains no integrated amylase gene. The resulting diploid cells were sporulated and dissected. The amylase secretion phenotype segregated 2:2, since only one copy of the amylase expression cassette was present in the parental diploid genome. If the mutation had been *cis*-acting, the sizes of all halos should have been large, however both small and large halo sizes were detected, indicating that the mutations were not linked to the integrated α-amylase gene (data not shown). Among 26 tetrads examined from the *pop2-1* cross, 4 were parental ditype, 6 were nonparental ditype and 16 were tetratype. Furthermore, when pMT56 (a 2-μm-based, *PGK*-amylase expression plasmid) was introduced into the cells from tetratype segregants showing no halos, one showed a large halo and the other showed a small halo (data not shown). In the case of *pop2*, temperature sensitivity for growth and large halo phenotype cosegregated (data not shown). These results indicate that the *pop2* mutation was *trans*-acting.

pop2 mutation causes temperature sensitivity for growth

Previously, we isolated the *rgr1* mutant which oversecretes mouse α-amylase under the control of the *SUC2* promoter (4). The *rgr1* cells exhibites temperature-sensitive growth (4, 5). So,we also examined temperature sensitivity for growth of the mutants isolates. Among the original mutant isolates, only the *pop2-1* strain was found to be temperature sensitive for growth (ts). The *pop2-1* cells, which contained amylase expression cassette, were crossed to wild-type cells and the resultant diploid cells were sporulated and dissected. The temperature sensitivity for growth segregated $2^+:2^-$, indicating that this defect was due to a mutation in a single gene. The amylase secretion phenotype segregated 2:2, as described in the *cis*- and *trans*-acting test, but

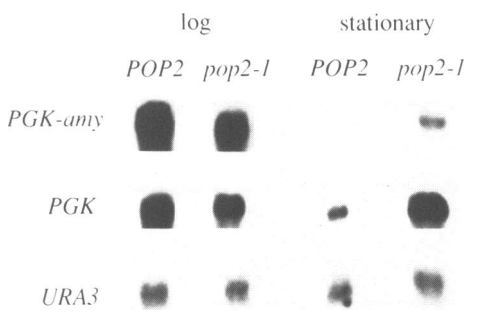

Figure 1. Northern analysis of *pop2* mutants. Total RNAs were prepared from cultures growing logarithmically (1×10^7 cells/ml) and stationary phase (three-day culture) in YPD media, and were electrophoresed, transferred to nitrocellulose filters and hybridized to the probe DNAs. The probe DNAs used were as follows. Amylase, 1.5 kb *Bam*HI fragment of pSAK031; *PGK*, 3 kb *Hin*dIII fragment of pB1 (Hitzeman, Clarke and Carbon, 1980); *URA3*, 1,565 bp *Nru*I-*Sma*I fragment of YIp5. All the probe DNAs were nick translated with DNA polymerase I and labeled with [α-^{32}P]dCTP. Left, logarithmically growing culture; right, stationary culture. *POP2*; A441 (wild-type), *pop2-1*; A417.

only ts cells showed larger halos. The dominance test indicated that this temperature sensitive phenotype was recessive (data not shown). This ts defect was linked tightly to the *pop2* mutation. During a series of four backcrossings to the parental strain, no segregation of ts, snf and amylase overproduction phenotypes was observed (P: NPD: T = 65: 0: 0). These results suggested that the temperature sensitivity for growth and the large size of the amylase halo were caused by a single mutation, *pop2*. Yeast cells carrying null alleles of *POP2* gene showed the same phenotypes (see below).

Carbon source utilization

We tested the utilization of carbon source in the *pop 2* mutant. Mutant cells grown in CSM were inoculated into complete synthetic medium containing various carbon sources (glucose, glycerol, lactose, ethanol or sodium acetate) and were cultivated for three days at 24°C. The *pop2* cells could not utilize any of the various carbon sources tested, with the exception of glucose. The *pop2* mutant cells also could not ferment sucrose under anaerobic conditions, demonstrating a *snf* (*s*ucrose *n*on*f*ermentor) phenotype.

Glucose regulation and Northern analysis

The activity of secreted amylase was assayed in mutants grown in YPD medium. The *pop2* mutants oversecreted amylase at levels 5- to 6-fold higher than the parent strain (Table 2). Since the *pop2* mutant exhibited a *snf* phenotype we decided to also examine invertase secretion. We observed that the activity of secreted invertase in the *pop2* cells was one-fourth that of the parental strain under glucose-derepressing conditions (Table 2). Since the *pop2* mutant could not ferment sucrose under anaerobic conditions it is possible that these observed levels of invertase activity are due to a transient derepression of *SUC2*.

To test whether the glucose-derepression resistance of *pop2* was specific for the *SUC2* gene the activity of isocitrate lyase, which is known to be glucose repressible, was assayed. We found that the activity of isocitrate lyase in the *pop2* cells was derepressed to only one-third of the level observed in the parental strains (Table 2). Next, the effects of the *pop2* mutation on transcription of *PGK*, *PGK*-amylase and *URA3* genes were analyzed by a quantitative Northern analysis (Figure 1). We observed little difference in the amounts of transcripts produced in wild-type or *pop2* cells harvested during log phase. However, transcription of the *PGK* and *PGK*-amylase genes was reduced drastically in stationary-phase cells (to almost undetectable levels) in wild-type cells, whereas that of the *URA3* gene did not change. In contrast to the wild-type cells, the amount of *PGK* and *PGK*-amylase gene transcripts in stationary-phase cells of the *pop2* mutant were found to be 5- to 10-times higher than those of the wild-type cells.

Table 2. Expression of various enzymes and contents of reserve carbohydrates

Relevant genotype	Invertase (U / ml / min)		Isocitrate lyase (U / mg protein / min)		Amylase (U / ml)	Glycogen (mg / mg protein)	Trehalose (mg / mg protein)
	R	D	R	D			
pop2	2.2	4.6	< 0.5	2.0	1.50	20	260
Wild type	0.7	13.7	< 0.5	7.3	0.27	73	1,000

The values are the average of determination for three independent experiments. R, repressed; D, derepressed

Table 3. Genetic mapping of *pop2-1* on chromosome XIV

Gene pair	No. of ascus type [a]			Map distance (cM) [b]
	PD	NPD	T	
pop2-SUP28	108	1	23	11.0
pop2-lys9	122	0	10	3.8
SUP28-lys9	43	0	10	9.4 [c]

[a]Abbreviations: PD, parental ditype; NPD, nonparental ditype; T, tetratype.
[b]Genetic map distances in centimorgans (cM) was calculated by the following equation: cM = [T + 6NPD)/2(PD + NPD + T)] × 100
[c]ref. 39

Amount of reserve carbohydrates and sporulation of *pop2* mutation

During the series of backcrossing, we found that the *pop2* cells showed reduced staining with iodine vapor, suggesting that this mutant contains less glycogen than wild-type cells. The reduced staining with iodine vapor and the *pop2* mutation.were tightly linked and cosegregated 2:2 in more than 80 tetrads. The amount of storage carbohydrates (glycogen and trehalose) were measured (Table 2). Glycogen and trehalose levels in the *pop2* cells were approximately 30% those of the wild-type cells. To test the effect of *pop2* on sporulation, a diploid homozygous for *pop2* was constructed. This diploid failed to sporulate even after 10 days on sporulation plates. The heterozygous diploid sporulated normally as described above.

Genetic interaction between *pop2* and *ssn6*

A suppressor of some *snf* mutations, *ssn6*, causes constitutive expression of the *SUC2* gene (25, 26). *SSN6* has recently been shown to be a general repressor of transcription in yeast (27). Accordingly, we tested the interaction between *pop2* and *ssn6*. Haploid cells carrying *pop2* and *ssn6* were constructed by standard genetic crossing and were used to test temperature

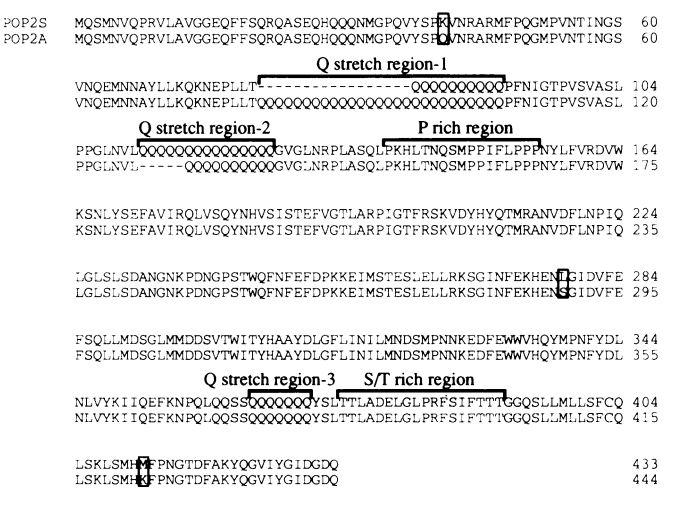

Figure 2. Comparison of amino acid sequence of the *POP2* gene from S288C strain (*POP2S*) and the *POP2* gene from A364A strain (*POP2A*). Amino acids are numbered on the right. Different amino acids between the two POP2 proteins are enclosed by rectangles.

sensitive growth and the *snf* phenotype. Although these doubly-mutant cells were unable to grow at 37°C they could ferment sucrose and invertase activity was fully derepressed under glucose-derepressing conditions, indicating that they were phenotypically snf$^+$.

Linkage study and genetic map position of *pop2*

Among the several markers tested, significant linkage was detected between *pop2* and *lys9* on chromosome XIV. Linkage between *pop2* and *SUP28* was also assessed (Table 3). *pop2* was mapped to a position 11.0 centimorgans distal to *SUP28* and 3.8 centimorgans distal to *lys9*. There is no mutation reported at this map position, thus *pop2* is a previously unreported locus.

Cloning the *POP2* gene

We cloned the *POP2* gene by transforming a *pop2-1 leu2* strain (A419) with a genomic library carried on the multicopy vector YEp213. Plasmids which complement *pop2-1* should restore the sucrose fermentor phenotype, cause a reduction of amylase halo and restore temperature-resistant growth (Ts$^+$). Selection was for the Ts$^+$ phenotype followed by the sucrose fermentor phenotype and reduction of amylase halo. Five complementing plasmid DNAs were obtained and all of them had the same 4 kb *Bam*HI fragment. A 2 kbp minimum complementing fragment (*Eco*RI-*Hin*dIII fragment of POP2A, see Fig. 4) was further subcloned in YEp213 (YEpPOP2) and YCp50 (YCpPOP2). Both plasmids suppressed all of the phenotypes caused by the *pop2-1*. Integration mapping suggested that the DNA fragment was tightly linked to the *POP2* locus (data not shown).

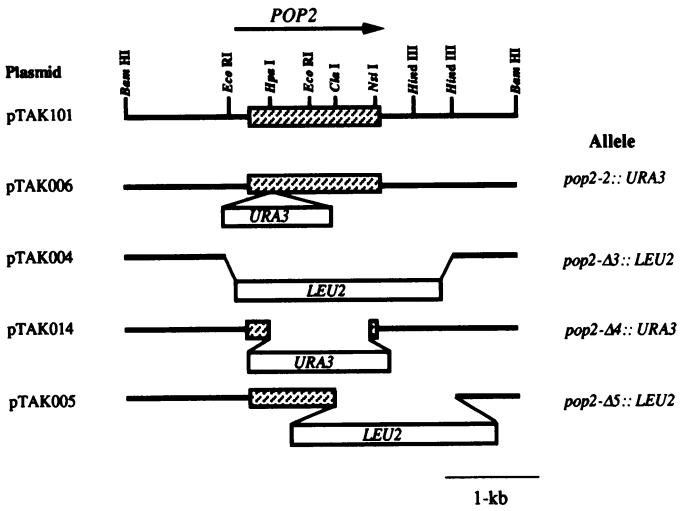

Figure 3. Disruption of *POP2* gene.

Sequence and analysis of the *POP2* gene

The nucleotide sequence of the *POP2* gene was determined and found to contain an open reading frame of 1,302 bp (433 amino acids) that could encode a 49,684-dalton protein. To identify the RNA encoded by *POP2*, poly(A)-containing RNA was analyzed by Northern hybridization. An approximately 1.4 kb RNA was detected (data not shown). Nuclease S1-mapping analysis showed that major initiation site of *POP2* transcript was −110 (data not shown). The predicted *POP2* protein had three glutamine-stretches, a proline rich region and a serine/threonine rich region (Fig. 2), which resembles many transcription factors, such as *SNF2* (28), *SNF5* (29), *GAL11* (30), *HAP1* (31), *HAP2* (32) and *SSN6* (33) (see review, ref. 34).

Location of *pop2-1* mutation

We cloned the *pop2-1* DNA from the strain A421 and determined the nucleotide sequence. Only C to T change in nucleotide 187 was found. This one-base change generated a stop codon, indicating that the original *pop2-1* mutation is a nonsense mutation. As shown in Fig. 3, we generated several mutant alleles of the *POP2* gene (*pop2-2*; insertion at 234, *pop2-Δ3*; a null mutation, *pop2-Δ4*; C-terminal deletion, and *pop2-Δ5*; an internal deletion). All of the strains carrying these mutant alleles were able to grow and showed the same phenotype as *pop2-1*, indicating that the *POP2* gene is not essential for cell viability.

Comparison of the *POP2* DNA sequence between S288C and A364A

We also isolated the *POP2* DNA from a yeast strain of a different genetic background, A364A using the 2 kb minimum complementing fragment from S288C strain as a probe. This allele was designated *POP2A*. It is well known that these two laboratory strains differ in the length of some chromosomes as analyzed by pulsed-field gel electrophoresis (35). The nucleotide sequence of *POP2A* was 33 bp longer and was 96% identical to that of *POP2S*. Some base changes resulted in a change in restriction site (Fig. 4), however, almost all nucleotide changes were silent. Major amino acid differences were as follows: 1) insertion of 16 glutamines at position 81, 2) deletion of 5 glutamines at position 128, 3) lysine^{41} to glutamine^{41}, 4) leucine^{278} to serin^{289} and 5) methionine^{412} to lysine^{423} (see

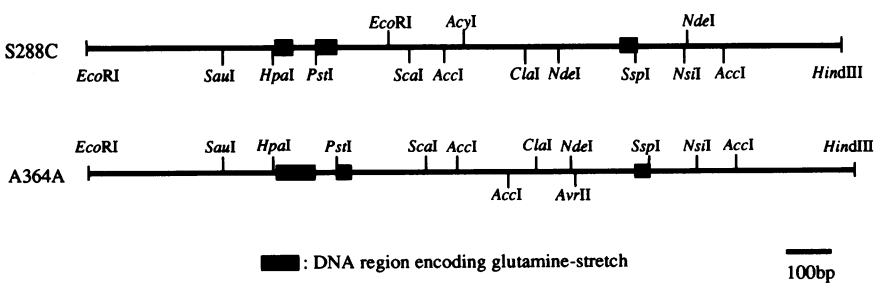

Figure 4. Restriction maps of *POP2* genes from S288C and A364A. The nucleotide sequence of the *POP2* gene from S288C (*POP2S*) and the *POP2* gene from A364A (*POP2A*) are appeared in the DDBJ, EMBL and GenBank with the accession number D12807 and D12808, respectively.

Fig. 2). The *POP2A* protein was composed of 444 amino acids. Both *POP2S* and *POP2A* carried on centromere plasmids suppressed all of the phenotypes caused by *pop2-Δ4* (C-terminal deletion).

DISCUSSION

We have isolated and characterized a new mutation, *pop2*, which is resistant to glucose derepression and temperature-sensitive for growth. This mutant was isolated as an overproducer of α-amylase in a strain containing the mouse α-amylase gene under the control of the *PGK* promoter. The *pop2* mutation was mapped to a location on chromosome XIV to which no other gene had been previously mapped; 11.0 centimorgans distal to *SUP28* and 3.8 centimorgans distal to *lys9* (Table 3). The *pop2* mutation affects various cellular functions, causing ; 1) temperature-sensitive growth, 2) resistance to glucose-derepression (Table 2), 3) defective sporulation of homozygous diploids and 4) reduced amounts of reserve carbohydrates (Table 2). These four phenotypes linked through several backcrosses. Northern analysis confirmed that this defect in glucose-derepression affects mRNA levels (data not shown). *Pop2* mutants display a *snf* phenotype (2, 36, 37), i.e. they are unable to utilize sucrose as a carbon source in the absence of oxygen. None of the previously isolated *snf* mutations map to chromosome *XIV*, indicating that *pop2* is a new *snf* mutation. Some snf mutations are suppressed by *ssn6*, a mutation which causes the constitutive expression of *SUC2* (25, 26). We observed that *ssn6* suppressed the *snf* phenotype of *pop2*, but not the temperature-sensitive growth defect. Thus the relationship between *pop2* and other *snf* mutations remains to be clarified.

The nucleotide sequence of the *POP2* gene contains a 1,302 bp open reading frame The predicted 49.7-kilodalton *POP2* protein encoded by this gene contains three glutamine-stretches, a proline rich region and a serine/threonine rich region (Fig. 2). Such sequences are commonly found in many factors which affect gene transcription, such as *MCM1*, *SSN6*, *GAL11*, *HAP2*, *PHO2*, *SNF2*, *SNF5*, *Antenapedia*, *Engrailed*, *Notch*, *Sp1*(see ref. 34). However, no obvious DNA-binding consensus sequence was found, suggesting that POP2 may affect transcription by interacting with other proteins. The most prominent feature of the POP2 sequence is the presence of several glutamine-rich regions. In case of SSN6 such glutamine-rich regions have been shown to be dispensable for its function (38). Perhaps these stretches served to physically separate functional domains within proteins. We determined that the *pop2-1* allele contained a nonsense mutation. Disruption of the *POP2* gene resulted in the original *pop2-1* mutant phenotypes. We also isolated the *POP2* gene (*POP2A*) from A364A cells which has a defferent genetic background from S288C. The nucleotide sequence of *POP2A* was 96% identical to that of *POP2S*. Some base changes resulted in a change in restriction site (Fig. 4) and in amino acid sequence (Fig.2), however, both *POP2S* and *POP2A* suppress all of the phenotypes caused by *pop2-Δ4*.

We found that steady-state amounts of *PGK* and *PGK-amylase* gene mRNAs in stationary-phase cells were 5- to 10-times higher in the *pop2* mutant than the wild-type cells. However, *URA3* mRNA levels remained unchanged (Fig. 1). This result suggests that in *pop2* cells glucose-derepression is limited to stationary phase, therefore POP2 does not appear to be required for general glucose derepression. Taken together with the defect in accumulation of storage carbohydrates, the results presented suggest that *POP2* is involved in some aspects of coordinating entry into stationary phase. Since the POP2 protein bears some resemblance to other factors which affect gene transcription we believe that the higher levels of *PGK* and *PGK-amylase* transcripts observed is probably the result of enhanced gene expression. Alternatively it is possible that *pop2* affects mRNA stability. Further study will be required to distinguish between these possibilities.

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