Isolation and Characterization of Mutants Which Show an Oversecretion Phenotype in *Saccharomyces cerevisiae*

Akira Sakai, Yuki Shimizu and Fumio Hishinuma

Labaratory of Molecular Genetics, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooyaa, Machida-shi, Tokyo 194, Japan

Manuscript received December 21, 1987
Revised copy accepted March 5, 1988

ABSTRACT

We have isolated mutants responsible for an oversecretion phenotype in *Saccharomyces cerevisiae*, using a promoter of SUC2 and the gene coding for a-amylase from mouse as a marker of secretion. These mutations defined two complementation groups, designated as *osel* (over secretion) and *rgrl* (resistant to glucose repression). The *osel* mutant produced an oversecretion of amylase by 12- to 15-fold under derepressing conditions; however, the amylase mRNA was present at nearly the same amount as it was in the parent cells. No expression of the amylase gene was detected under repressing conditions. The *rgrl* mutant oversecreted amylase by 11- to 13-fold under repressing conditions and by 15- to 18-fold under derepressing conditions. The *rgrl* mutant showed pleiotropic effects on the following cellular functions: (1) resistance to glucose repression, (2) temperature-sensitive lethality, (3) sporulation deficiency in homozygous diploid cells, and (4) abnormal cell morphology. The *rgrl* mutation was not allelic with *snf6* and *yc9*, and failed to suppress *snf1*.

THE yeast *Saccharomyces cerevisiae* is a good host for heterologous gene expression and protein secretion. Many proteins have been shown to be produced by this species and are also secreted readily, examples being epidermal growth factor (BRAKE et al. 1984), β-endorphin (BITTER et al. 1984) and calf prochymosin (SMITH, DUNCAN and MOIR 1985). A requirement for the secretion of protein is the presence of signal sequences, which lead the preproproteins into the endoplasmic reticulum, and which are necessary in order for a specific secretion pathway to be followed. The secretion pathway in *S. cerevisiae* is well characterized through the analysis of sec (secretion deficient) mutants (SCHENKMAN and NOVICK 1982); however, the precise molecular mechanism of this secretion pathway remains to be established. In order to study the exact mechanism of protein secretion and to attempt to produce heterologous proteins, the use of mutants which exhibit oversecretion phenotypes as well as the *sec* mutants, could provide powerful investigative tools. The mutants showing an oversecretion phenotype, isolated through the use of heterologous gene expression, may be classified into three categories: mutations in cis-acting promoter regions (class I), mutations in trans-acting genes (class II) and post-transcriptional mutations (class III). Supersecreting mutants showing a general oversecretion phenotype have been isolated using a SUC2 promoter and calf prochymosin genes as markers (SMITH, DUNCAN and MOIR 1985), but the characterization of these mutants has not been reported as yet.

In this paper, we describe the isolation and an initial characterization of mutants which show an oversecretion phenotype, using the SUC2 promoter and mouse a-amylase as markers. These mutations belong to classes II and III as defined above.

MATERIALS AND METHODS

**Strains:** All yeast strains used in this study were isogenic or congenic with strain S288C (MATa, SUC2, gal2). The genotypes and sources of the strains are listed in Table 1. Crosses, sporulation and tetrad dissection were performed as described by MORTIMER and HOWTHORNE (1969) and SHERMAN, FINK and LAWRENCE (1978). *Escherichia coli* strain HB101 was employed as the host for propagating plasmids.

**Media:** The basic culture medium used for *S. cerevisiae* was a YPD medium containing 1% Bacto-yeast extract, 2% Bacto-peptone and 2% dextrose. A YP sucrose (YPsuc) medium containing 1% Bacto-yeast extract, 2% Bacto-peptone and 2% sucrose was used in testing the yeast for an ability to ferment sucrose. The media were solidified with 2% Bacto-agar for plates. Scoring for the fermentation of glucose or sucrose was carried out under anaerobic conditions in a Gaspak disposable Anaerobic System (BBL). For detecting the activity of secreted mouse a-amylase, YP starch (YPst) plate containing 1% Bacto-yeast extract, 2% Bacto-peptone, 2% soluble starch (Merck) and 2% Bacto-agar was used. TUP plate containing 0.15% Bacto-yeast extract, 1% Bacto-peptone, 2% dextrose, 6 mg/ml sulfanilamide, and 50 μg/ml dTMP was prepared according to the method of WICKNER (1974). Luria-broth was used for the culture of *E. coli* and this was supplemented with ampicillin for selection of the transformants as described by MANIATIS, FRITSCHE and SAMBROOK (1982).

**Transformations:** The transformation of *E. coli* was performed as described by HANAHAN (1983). The transformation of yeast was performed as described by ITO et al. (1985).

**Construction of an integrative secretion vector of mouse a-amylase and the isolation of its single-copy integrant cells:** The yeast multicopy secretion vector pSMF38
TABLE 1
List of yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A192</td>
<td>MATa ars7 can1 leu2</td>
<td>This work</td>
</tr>
<tr>
<td>A192/pSAKO11/MluI</td>
<td>MATa ars7 can1 leu2</td>
<td>This work</td>
</tr>
<tr>
<td>A258</td>
<td>MATa osel-1 can1 his4</td>
<td>This work</td>
</tr>
<tr>
<td>A260</td>
<td>MATa osel-1 can1 his4</td>
<td>This work</td>
</tr>
<tr>
<td>A285</td>
<td>MATa tyr1-1 ara7 asp5</td>
<td>This work</td>
</tr>
<tr>
<td>A287</td>
<td>MATa tyr1-1 asp5 leu2</td>
<td>This work</td>
</tr>
<tr>
<td>MCY1489</td>
<td>MATa ynt/L-3 ade2-101</td>
<td>M. CARLSON</td>
</tr>
<tr>
<td>MCY1529</td>
<td>MATa ssn6-5::URA3</td>
<td>M. CARLSON</td>
</tr>
</tbody>
</table>

* A single copy integration of pSAKO11 linearized with MluI at SUC2 gene.

(NISHIZAWA, OZAWA and HISHINUMA 1987), constructed through the use of the promoter and the signal sequence of yeast invertase coded by the SUC2 gene, was digested with EcoRI and BamHI and the resultant 884-bp DNA fragment containing the promoter and the signal sequence of the SUC2 gene was isolated. The 553-bp DNA fragment bearing the SUC2 terminator was also digested by digesting pSMF38 with BamHI and HindIII. These two DNA fragments were ligated with EcoRI and HindIII digested from YIp5 whose own signal sequence was deleted, and the resultant plasmid, pSAKO10, is the yeast integrative secretion-vector using the SUC2 promoter; it has a single BamHI site after the signal sequence. The pSAKO10 plasmid was digested with BamHI and ligated in frame with a mouse α-amylase gene whose own signal sequence was deleted, and the resultant plasmid was designated pSAKO11. To secrete human β-endorphin, the human pro-β-endorphin gene (TAKAHASHI et al. 1981) was inserted into pSMF38 at the BamHI site (pSAKO25).

pSAKO11 had a single StuI (AatII) site at the URA3 gene, a single MluI site at the SUC2 promoter and a single AatII site at the pBR322 region. The pSAKO11 DNA was linearized with StuI or MluI. YNN27 cells were transformed with linearized pSAKO11 by the lithium acetate method (ITO et al. 1989) and chromosomal DNA was extracted from the transformants as described by SHERMAN, FINK and LAWRENCE (1978). The chromosomal DNA was digested with StuI, MluI or AatII and electrophoresed in an agarose gel. The single copy integrants were selected by Southern hybridization as described by CARLE and OLSON (1985).

Mutagenesis and isolation of mutants: The single copy integrant of pSAKO11 DNA (YNN27/pSAKO11/MluI) was treated with 3% ethyl methanesulfonate (EMS) as described by SHERMAN, FINK and LAWRENCE (1978) and the time of exposure to the mutagen was adjusted to yield a survival rate of 45–55%. The EMS-treated cells were grown on YPD plates at 24°C for three days. The established colonies were patched on a YP starch (YPSt) plate and incubated overnight at 24°C. The activity of secreted amylase was detected by irrigating the colonies on the YPS plates with a solution of 0.2% iodine in 0.2% potassium iodide, as described by NISHIZAWA, OZAWA and HISHINUMA (1987). The colonies showing larger halos than were observed in the original transformants were selected as mutants. The sizes of the halos of putative mutants were tested three times and the mutants were backcrossed to the parental strains at least five times.

The temperature sensitivity underlying the growth of the cells was determined from their ability to grow on YPD plates at 37°C.

Complementation analysis: To test pairs of mutations for complementation, heterozygous diploids were constructed and isolated by prototrophic selection. The size of the halos caused by amylase of the diploids were determined on the YPS plates.

DNA manipulations: The digestion of DNA with restriction enzymes, ligation, kinasing and the preparation of plasmid DNA were performed as described by MANIATIS, FRITSCH and SAMBROOK (1982).

S1 nuclease analysis: Total cellular RNA was isolated according to the method of STRUHL and DAVIS (1981), with the exception that zymolyase, at a final concentration of 20 units per ml was used instead of lyticase. S1 mapping was performed as described by MANIATIS, FRITSCH and SAMBROOK (1982). The total RNA isolated from the yeast was hybridized with the DNA fragment probe in the presence of 80% (v/v) formamide, for 4 hr at 42°C. Nonhybridized DNA was digested with S1 nuclease at 37°C. The protected hybrid fragments were subjected to electrophoresis on an 8% sequencing gel.

Enzyme assays: The activity of secreted amylase in the culture medium was determined as described by BERFIELD (1953). The glucose-repressed and -derepressed cells were prepared as described by NISHIZAWA, OZAWA and HISHINUMA (1987). In short, log-phase cells (1 x 10^6 cells/ml) were washed once with YP medium containing 5% dextrose (YPD5%) for glucose-repressed cells or YPD0.2% for glucose-derepressed cells. The cells were resuspended with YPD5% or YPD0.2% at cell density of 1 x 10^6 cells/ml and cultivated at 24°C. Under these conditions, glucose in the derepressed culture medium was exhausted after 2 hr of incubation. The glucose-derepressed cells were harvested after further 3 hr of cultivation. The activity of invertase was determined in whole cells as described by GOLDSTEIN and LAMPEN (1975) and by CELENZA and CARLSON (1984b). The activity of isocitrate lyase was determined in crude extracts according to the method of DIXON and KORNBERG (1959). The concentrations of protein were determined by the dye-binding method of BRADFORD (1976). The amount of β-endorphin-containing peptide in the culture supernatant was assayed using a radioimmunoassay kit from New England Nuclear.

RESULTS

Isolation of mutants: To isolate the oversecretion mutants, direct screening for the oversecretion phenotype is a convenient procedure, since this method...
makes it possible to select mutations which affect the steps in both transcription and secretion. To this end, we have used the SUC2 promoter and its signal sequence for transcription and secretion, and used mouse α-amylase as a marker.

Mutants which showed an oversecretion phenotype were selected as described in MATERIALS AND METHODS. Each mutant which was originated from YNN27/pSAKO11/MluI was crossed to A192/pSAKO11/MluI 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 (Figure 1). YNN27/pSAKO11/MluI and A192/pSAKO11/MluI were both integrants of pSAKO11 at SUC2 gene on chromosome IX. Only those mutants in which the presence of a large halo phenotype could be attributed to a single mutation, were characterized further. Five mutants were recovered among 3500 colonies screened. Each mutant showed no difference from the parental strain (YNN/pSAKO11/MluI) with respect to the utilization of carbon source and requirements for nutrition. Only one mutant (rgr1) showed a temperature-sensitive lethality (see below). Each mutant was crossed to A192/pSAKO11/MluI, and the resulting diploids were tested for the sizes of their halos on YPS plates. All five diploids showed halo sizes comparable to diploid from cross of parent (YNN/pSAKO11/MluI) to A192/pSAKO11/MluI, indicating that these mutations were recessive.

**Complementation analysis:** Complementation analysis was performed as described in MATERIALS AND METHODS based on the size of the halos on YPS plates. Two complementation groups were identified (data not shown). The first complementation group, designated ose1 (oversecretions), included four mutations (ose1-1, ose1-2, ose1-3 and ose1-4) which complemented rgr1 but failed to complement each other. The ose1-3 and ose1-4 mutations were both leaky and these were not characterized further. The second complementation group, designated rgr1 (resistant to glucose repression), included only one mutation (rgr1-1) which complemented the ose1 mutation.

**cis- and trans-acting test:** To determine whether the mutations were cis-acting or trans-acting, the ose1-1 and the rgr1-1 strains were crossed with A192, which had no amylase integration. The resulting diploids were sporulated and dissected. The amylase secretion phenotype segregated 2:2. If the mutation was cis-acting, the sizes of all halos should have been large. Figure 2 shows, however, that both small and large halo sizes were detected, indicating that the mutations were independent of the integration of amylase. Among 37 tetrads examined for the ose1-1 cross, six tetrads were parental ditype (two showed large halos and two showed no halo), nine were nonparental ditype (two showed small halos and two showed no halo) and 22 were tetraplotype (one showed large halo, one showed small halo and two showed no halo). In the case of rgr1-1, 38 tetrads were examined and six were parental ditype, six were nonparental ditype and 26 were tetraplotype. These results indicate that the mutations both were trans-acting.

**Linkage studies of ose1 and rgr1:** The analyses of crosses including the centromere-linked markers trpl, met14, leu2, aro7 and alleles of ose1 and rgr1, showed that a majority of the tetrads were tetraplotype. None of the genes is linked tightly to any centromere. There was no tight linkage to canl, his4, SUC2, MAT, snf1 or snl6. We have recently isolated cloned DNA fragment that complements the rgr1 mutation. This DNA fragment hybridized with chromosome XII on the orthogonal-field-alternation gel, suggesting that the rgr1 localizes on chromosome XII (in preparation).

**Glucose regulated secretion:** The activity of secreted amylase was assayed in the mutants grown under glucose-derepressing conditions. Both mutants oversecreted amylase by 12- to 18-fold, compared with the parent strain (Table 2). The time course experiment suggested that the ose1 strain did not secrete amylase under glucose-repressing conditions, but that the rgr1 strain did secrete amylase constitu-
shown). The contrast the respective under glucose-repressing conditions (data not shown). The rgrl mutation is believed to affect the glucose regulated expression of the SUC2 gene. To test this possibility, the mRNA synthesized in vivo was analyzed through the use of quantitative S1 nuclease mapping. The 5' end of the EcoRI site in the amylase gene was labeled and used as a probe for the S1 mapping, as shown in Figure 3. In rgrl strain, the amylase mRNA was transcribed under both glucose-repressing and -derepressing conditions (Figure 3, lanes 4 and 7). The amount of the mRNA was five to ten times more than that expressed by the parent cells under derepressing conditions. The mRNA start point was identical in each case, and was identical to the start point (-40) in the SUC2 gene as reported by CARLSON et al. (1983). The other transcripts which had different start points could not be detected, in any case.

Differing from rgrl, the amount of mRNA synthesized in the osel strain was nearly the same amount as was that of the parent cells, even though the secreted amylase was 12- to 15-fold greater than the parent cells. This may indicate that osel affects some steps after transcription.

Since it is difficult to quantitate the activity of amylase in the presence of high concentrations of glucose, we introduced a multicopy plasmid pSAK025 which allowed the secretion of human pro-β-endorphin under the control of the SUC2 promoter, into the mutant strains. Under glucose-derepressing conditions, the osel strain and the rgrl strain oversecreted β-endorphin by six- to sevenfold and by 11- to 12-fold, respectively. The osel strain did not secrete β-endorphin under repressing conditions, but by contrast the rgrl strain did secrete β-endorphin under repressing conditions. These results also suggested that the oversecretion by the mutants was not specific for amylase.

If the rgrl mutation causes a glucose repression resistance, then the activity of invertase (SUC2 product) should be detectable under repressing conditions in the rgrl strain. As was expected, the activity of invertase in the rgrl strain was detected under glucose repressing conditions: this activity was one-fourth that observed under fully derepressing conditions (Table 2).

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Amylase (units/10^7 cells)</th>
<th>Invertase (units/10^7 cell)</th>
<th>Isocitrate lyase (units/mg)</th>
<th>β-Endorphin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rgrl (SUC2::pSAKO11)</td>
<td>R: 7.07</td>
<td>D: 4.6</td>
<td>R: 3.14</td>
<td>D: 4.54</td>
</tr>
<tr>
<td>osel (SUC2::pSAKO11)</td>
<td>R: 5.70</td>
<td>D: 2.3</td>
<td>&lt;0.5</td>
<td>3.92</td>
</tr>
<tr>
<td>Wild type</td>
<td>R: 0.41</td>
<td>D: 3.6</td>
<td>&lt;0.5</td>
<td>3.31</td>
</tr>
</tbody>
</table>

Secreted invertase activity was assayed in whole cells. Secreted amylase activity and β-endorphin were assayed in medium. Isocitrate lyase activity was assayed in crude extract. The values are the average of determination for three independent experiments. R, repressed; D, derepressed.

* Due to high concentration of glucose which inhibits amylase activity, secreted amylase activity was not assayed.

TABLE 2

Expression and secretion of various enzymes and proteins

![Figure 3.—Analysis of mRNA from the SUC2-amylase chimeric gene. Samples of glucose-repressed cells were taken from cultures growing exponentially in YP media containing 5% glucose (repressing conditions), and derepressed cells were obtained by shifting such cells to YP media containing 0.2% glucose (derepressing conditions) for 3 hr. The probe was hybridized total cellular RNAs (20 μg) isolated from glucose-repressed and -derepressed cells of the wild, osel and rgrl strains, and was treated with S1 nuclease (60 units) for 30 min. Protected DNA fragments were analyzed on an 8% polyacrylamide sequencing gel. The probe was prepared as follows. The pSAK011 DNA was digested with EcoRI and the 5'-ends were labeled with [γ-32P]ATP and T4 polynucleotide kinase. The labeled DNA was digested with MluI and a 750-bp DNA fragment was isolated as the probe. The pBR322 DNA was digested with HinfI and, at the 5'-end was labeled with [γ-32P]ATP and T4 polynucleotide kinase, used as a size marker. 1, 8 = pBR322 HinI marker; 2 = derepressed wild-type cells; 3 = derepressed osel cells; 4 = derepressed rgrl cells; 5 = derepressed rgrl cells; 6, 7 = repressed osel cells; 7 = repressed rgrl cells. E, EcoRI; M, MluI. |
Yeast Oversecretion Mutants

37°C

1 0.2 0.3 0.4 0.5

0.

0.7

b

FIGURE 4.—Temperature sensitive lethality of rgr1. a. Temperature sensitive lethality. A287 (rgr1) cells were crossed to A192 pSAK011/MnuI and the resulting diploid cells were sporulated and microdissected. The germinated tetrads were patched on YPD plates and incubated at 24° or 37° for 3 days. b. Amylase halo assay. The tetrads were patched on YPS plates and incubated at 24°. Secreted amylase activity was detected as a halo. 1–8 = number of tetrads.

To test further the possibility that the glucose-repression resistance of rgr1 was specific for the Suc2 promoter, the activity of isocitrate lyase, which is known to be glucose repressible, was assayed. The activity of isocitrate lyase was repressed in the osel strains and in the parent cells under repressing conditions. On the contrary, the rgr1 mutation allowed the expression of the isocitrate lyase under repressing conditions. These results indicate that the rgr1 mutant was resistant to glucose-repression.

rgr1 mutation caused temperature-sensitive lethality: We have examined temperature-sensitive lethality of the mutants. To date, there have been no reports of temperature-sensitive lethal mutations which cause glucose repression resistance. Among the original mutant isolates, only the rgr1-1 strain showed temperature-sensitive lethality.

Figure 4a shows that the temperature-sensitive lethality segregated 2–:2–, indicating that the defect was due to a mutation in a single nuclear gene. The dominance test indicated that this temperature-sensitive lethality was recessive (data not shown). This defect was linked tightly to the rgr1 mutation (Figure 4b). During the series of five backcrossings to the parental strain, no segregation of these two phenotypes was observed (P:NPD:T = 97:0:0). These results suggest that the temperature-sensitive lethality and glucose repression resistant Suc2 transcription were caused by the single mutation, rgr1.

Interaction between rgr1 and snf1: Among the suppressor of snf mutations (sucrose non fermenter), only ssn6 causes constitutive (glucose repression in-
sensitive) expression of the SUC2 gene (CARLSON et al. 1984; SAROKIN and CARLSON 1985). Accordingly, we have tested the allelism between rgrl and ssn6. The rgrl strain was crossed to the ssn6 mutation, and the resulting diploid cells were able to grow at 37°C, showing a halo size comparable to that of the parental haploid strain. Therefore, rgrl is not allelic with ssn6.

Next, we examined the effect of the rgrl mutation on the snf1 mutation. The SNF1 gene which encodes a protein kinase is considered to play a central role in the carbon catabolite repression of S. cerevisiae (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984; Celenza and CARLSON 1984a, 1986). The rgrl strain was crossed to an snf1 mutant strain, and the resulting diploids were able to grow at 37°C and were shown to ferment sucrose. The diploid cells were sporulated, dissected and the germinated spores were used for testing temperature-sensitive lethality and the ability to ferment sucrose. Table 3 shows that rgrl does not suppress the sucrose-nonfermenting phenotype caused by snf1, nor does snf1 suppress the temperature-sensitive lethality caused by rgrl.

**Effect of rgrl mutation on cell morphology:** The carbon catabolite repression deficient mutation cye9*, allelic with tup1, urs7 and fkh1, shows morphological aberrations and bisexual mating in MATα strains (ROTHSTEIN and SHERMAN 1980; LEMONTT, FUGIT and MACKay 1980; STARK, FUGIT and MOWSHOWITZ 1980). To characterize the rgrl mutation, the pleiotropic effect of the mutation was determined. The rgrl mutation did not show a Tup− phenotype nor a mating abnormality; however, homozygous diploids for rgrl failed to sporulate. The rgrl strains showed chumpiness, while the morphology of the rgrl strain was highly abnormal, both in haploid (MATα and MATα) and diploid cells at permissive temperature (24°C) (Figure 5).

**DISCUSSION**

In order to isolate mutants featuring an oversecretion phenotype, one would consider necessary a method enabling the direct screening of the phenotype. We have already reported that mouse α-amylase is secreted into media under the control of SUC2 promoter, when the SUC2-amylase chimeric plasmid is introduced into yeast cells (NISHIZAWA, OZAWA and HISHINUMA 1987). The SUC2-amylase system could be useful for isolating mutants because the amylase secreted is detected easily as a halo on plates.

The chimeric gene for SUC2 promoter of yeast and α-amylase of mouse was integrated into yeast chromosomes, because the chromosomal-integrated construction is stable mitotically and meiotically, and is useful for genetic manipulation, as suggested by SMITH, DUNCAN and MOIR 1985. In some cases, chromosomal integration results in a higher expression than for multicopy plasmids (SMITH, DUNCAN and MOIR 1985). We did not find such an effect in our experiments, however. To reduce the possibility of the intragenic recombination causing a loss, or a duplication of the integrated gene, a single copy integrant was selected for further experiments.

Among the 3500 colonies which were screened after EMS mutagenesis, we obtained five strains exhibiting the oversecretion phenotype. We have isolated two recessive mutations (osel and rgrl) which caused the oversecretion of mouse α-amylase, under the control of SUC2 promoter.

The osel mutant secreted α-amylase 12–15 times more than the parent cells; however, it did not show the other defects, including carbon source utilization and temperature-sensitive lethality. This oversecretion phenotype did not arise from the increase in transcription, because the level of amylase mRNA was almost the same as that in the parent cells. Furthermore, transcription of the amylase gene is regulated by glucose and there was no transcription detected under glucose repressing conditions. When human β-endorphin was used as a marker for secretion, the osel mutant showed a tenfold oversecretion of β-endorphin under SUC2 promoter, indicating that the oversecretion phenotype was not specific for amylase. Three supersecreting mutations which belong to the class III mutation (see introduction) have been isolated using calf prochymosin as a marker. One of them is dominant (SSCX) and the other two are recessive (ssel and ssc2) (SMITH, DUNCAN and MOIR 1985). The expression levels of prochymosin in all mutants are comparable to wild-type cells and the only change is in the distribution of prochymosin between the vacuole and the medium (SMITH and GILL 1985). These supersecreting mutants have not yet been analyzed genetically and so the relationship between osel and supersecreting mutations remains unknown. The interaction between the osel and the sec mutations is currently under investigation. The OSE2 gene could be a negative regulator of some steps after transcription, and it could therefore belong to a class III type of mutation.

The rgrl mutant showed an increased level in SUC2-amylase chimeric gene transcription resulting in the amylase oversecretion phenotype and belonging to the class II type of mutation. This transcription was resistant to glucose repression. As well, rgrl mutation allowed the expression of isocitrate lyase under glucose repressing conditions. These results suggest that the rgrl mutant is resistant to glucose repression. While the rgrl mutant also showed a resistance to glucose repression, the effect of glucose derepression could still be observed.

The rgrl mutant exhibited a temperature-sensitive lethality. The oversecretion phenotype and the temperature-sensitive lethality are linked with each other.
tightly, and no segregation of these two phenotypes could be detected in 97 tetrads. We have not ruled out absolutely the possibility that the oversecretion phenotype and the temperature-sensitive lethality are caused by two independent mutations. However, a 8-kb DNA fragment which complements the oversecretion phenotype of \textit{rgr1}, also complemented the temperature-sensitive lethality (in preparation). This result suggested strongly that a single nuclear mutation, \textit{rgr1}, is responsible for two different phenotypes: namely resistance to glucose repression and temperature-sensitive lethality. Several mutations which affect either glucose repression or catabolite repression have been reported, such as \textit{ssn6} (NEIGEBORN and CARLSON 1984), \textit{tup1} (cy9) (LEMONTT, FUGIT and MAC\textit{K}AY 1980; SCHAMHART, TEN BERGE and VAN DE POLL 1975), \textit{ask2} (ENTIAN 1980, 1981), \textit{reg1} (MATSUMOTO, YOSHIMATSU and OSHIMA 1983) and \textit{cid1} (NEIGEBORN and CARLSON 1987). The \textit{rgr1} mutation was not allelic with \textit{ssn6} and failed to suppress \textit{snf1} and \textit{ssnfl} did not suppress the temperature-sensitive lethality of \textit{rgr1}. The \textit{rgr1} mutant cells did not show mating abnormality nor did they display utilization of exogenous deoxystreptamine monophosphate, as does the \textit{tup1} mutant (LEMONTT, FUGIT and MAC\textit{K}AY 1980). The \textit{reg1} (\textit{hex2}) mutation is linked tightly to \textit{trpl} (MATSUMOTO, YOSHIMATSU and OSHIMA 1983); however, \textit{rgr1} was not linked to \textit{trpl} tightly, nor to other centromere markers. The most important point is that none of the mutations showed temperature-sensitive lethality except \textit{rgr1}. Among the mutations which are involved in glucose regulation, only one which causes temperature-sensitive lethality has been reported, and that is \textit{ssn20} (NEIGEBORN, RUBIN and CARLSON 1986). The \textit{ssn20} mutation which is allelic with \textit{spf6} and is mapped on the right arm of chromosome \textit{VII} (NEIGEBORN, CLELENZA and CARLSON 1987; CLARK-ADAMS and WINSTON 1987) does not affect glucose regulation of the expression of \textit{SUC2}. The \textit{rgr1} was suggested to be mapped on chromosome \textit{XII}, indicating that it is a new temperature-sensitive mutation which affects the regulation of glucose. The interactions between \textit{rgr1} and other mutations remain to be elucidated.

We thank M. CARLSON and N. GUNGE for providing \textit{S. cerevisiae} strains, M. NHISHIZAWA for discussion, P. HICKS for critical reading of the manuscript and M. MATSUMOTO for typing the manuscript.

This work was supported by the Research and Development Project of Basic Technologies for Future Industries from the Ministry of International Trade and Industries of Japan.

LITERATURE CITED

BERRFELD, P., 1955 Amylase, \textalpha{} and \textbeta{} Methods Enzymol. 1: 149–158.


NEIGEBORN, L., J. L. CLELENZA and M. CARLSON, 1987 \textit{SSN20} is


