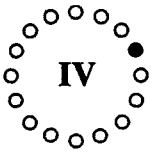
YEAST VOL. 10: 1211–1216 (1994)



Yeast Sequencing Reports

Nucleotide Sequence of the *SAC2* Gene of *Saccharomyces cerevisiae*

RALF KÖLLING*, ANGELA LEE, ELLSON Y. CHEN† AND DAVID BOTSTEIN‡

Department of Cell Genetics, Genentech, Inc., South San Francisco, CA 94305, U.S.A.
‡Department of Genetics, Stanford University School of Medicine, U.S.A.

Received 11 February 1993; accepted 28 March 1994

A temperature-sensitive mutation (*act1-1*) in the essential actin gene of *Saccharomyces cerevisiae* can be suppressed by mutations in the *SAC2* gene. A cloned genomic DNA fragment that complements the cold-sensitive growth phenotype associated with such a suppressor mutation (*sac2-1*) was sequenced. The fragment contained an open reading frame that encodes a 641 amino acid predicted hydrophilic protein with a molecular weight of 74 445. No sequences with significant similarity to *SAC2* were found in the GenBank and EMBL databases. A *SAC2* disruption mutation was constructed which had phenotypes similar to the *sac2-1* point mutation. A haploid *SAC2* disruption strain failed to grow at low temperature and the disruption allele suppressed the temperature-sensitive *act1-1* growth defect. The suppression phenotype was dependent on the strain background. The *SAC2* sequence has been submitted to the EMBL data library (Accession Number Z29988).

KEY WORDS — Actin; cytoskeleton; *SAC2*.

INTRODUCTION

Major conserved elements of the eukaryotic cytoskeleton have been identified in yeast, among them actin, the main constituent of microfilaments (Gallwitz and Seidel, 1980; Ng and Abelson, 1980). Immunofluorescence microscopy of yeast cells reveals asymmetrically arranged actin cortical patches and actin cables (Adams and Pringle, 1984; Kilmartin and Adams, 1984). The cortical actin patches are predominantly associated with growing regions of the cell, like the bud, while the filamentous actin cables are mainly visible oriented along the mother-bud axis of the non-growing portions of the cell. This arrangement of actin structures suggests an involvement of actin in polarized membrane growth and secretion.

*Present address and corresponding author: Institut für Mikrobiologie, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany.
†Present address: Applied Biosystems, Inc., Foster City, CA, U.S.A.

CCC 0749–503X/94/091211–06

Temperature-sensitive (ts) alleles of the single essential yeast actin gene have been constructed by *in vitro* mutagenesis (Shortle *et al.*, 1982, 1984), opening the way for a genetic analysis of the actin cytoskeleton. Pseudo-revertants of the ts *act1-1* mutant were isolated to identify factors which interact with actin (Adams *et al.*, 1989; Novick *et al.*, 1989). We are studying a series of recessive suppressor mutations (*sac*) that are cold sensitive (cs) for growth in addition to being able to suppress the ts growth phenotype of *act1-1*. We report here the sequence of the *SAC2* gene and the phenotypes of a *SAC2* disruption mutation.

MATERIALS AND METHODS

Strains, media and DNA manipulations

The yeast strains used in this paper are listed in Table 1. Descriptions of the genetic manipulations and the media used can be found in Sherman *et al.*

Table 1. Yeast strains.

Strain	Genotype
DBY1707	*MAT***a**/α *leu2-3,112/leu2-3,112 ura3-52/ura3-52 lys2-801/+*
DBY1918	*MAT***a** *sac2-1 ura3-52*
DBY5381	*MAT***a** *leu2-3,112 lys2-801 Δsac2::LEU2*
DBY5393	*MAT***a**/α *leu2-3,112/leu2-3,112 ura3-52/ura3-52 lys2-801/+ Δsac2::LEU2/+*
DBY5395	*MAT*α *act1-1 ade2 leu2-3,112 TUB2::URA3 ura3-52*

(1974). Cold-sensitivity of *sac* mutants was assessed at 11, 14 and 16°C. Plates were incubated up to 10 days at 11°C. Temperature-sensitivity of *act1-1* and suppression by *SAC2* mutations were scored after 3 days at 37°C. Growth tests were performed by spotting suspensions of cells in water onto plates using a 32-point inoculator. Standard procedures were used for DNA manipulations (Maniatis *et al.*, 1982). The following subfragments of the *SAC2* plasmid pRB397 were cloned into the vector YCp50 (Rose *et al.*, 1987). pRK4: 4·3 kb *Cla*I-*Sal*I; pRK5: 2·4 kb *Cla*I-*Eco*RI; pRK6: 3·2 kb *Bgl*II-*Sal*I; pRK7: 3·8 kb *Eco*RV-*Sal*I. Sequences 276 bp upstream of the *Sal*I site are derived from YCp50 (*Bam*HI-*Sal*I).

Disruption of the SAC2 *gene*

The plasmid pNB280 (provided by Peter Novick) was used for the construction of a *SAC2* deletion. pNB280 had been obtained by cloning the 4·3 kb *Cla*I-*Sal*I fragment, which is equivalent to the insert in pRK4, into the integrating vector YIp5. The 8·5 kb *Bgl*II-*Pvu*II fragment was isolated after digestion with *Bgl*II and partial digestion with *Pvu*II and ligated to the 2·5 kb *LEU2* fragment of pRB684 cut with *Bam*HI and *Sal*I. The *Sal*I end had been made blunt by treatment with Klenow polymerase. The plasmid pRK23 was recovered where the 1·6 kb *Bgl*II-*Pvu*II fragment of the *SAC2* gene is replaced by the *LEU2* marker gene. Transcription of *LEU2* is in the opposite direction to *SAC2*. This plasmid was cut with *Pst*I and transformed into the diploid yeast strain DBY1707 by the method of Ito *et al.* (1983). Five Leu^+ transformants were analysed by southern hybridization. *Eco*RI-digested chromosomal DNA was probed with a 1·3 kb *Eco*RI *SAC2* fragment. All five transformants were identical in that they showed, in addition to the 1·3 kb wild-type band, a 2·1 kb band which is the size expected for the fragment from the *SAC2* locus with the integrated *LEU2* marker gene (Figure 1B).

Sequencing of the SAC2 *gene*

The 3·8 kb *Eco*RV-*Sal*I fragment was subcloned into M13 derivatives (Messing, 1983) and sequenced by standard dideoxy sequencing methods (Sanger *et al.*, 1977).

RESULTS AND DISCUSSION

The *SAC2* gene had been cloned by complementation of the cs phenotype of the *sac2-1* mutant (Novick *et al.*, 1989). In order to localize the *SAC2* gene on the 9·7 kb insert of the original plasmid isolate (pRB397), fragments were subcloned into the low-copy centromere-containing vector YCp50. These subclones were used to transform the *sac2-1* mutant strain DBY1918 and tested for their ability to complement the cs defect of the *sac2-1* mutant (Figure 1A). We sequenced the smallest insert that gave rise to cold-resistant transformants, a 3·8 kb *Eco*RV-*Sal*I fragment. The sequence contained an open reading frame with the capacity to code for a 74 kDa hydrophilic protein and part of another reading frame (Figure 2). The complementation data in Figure 1A show that the longer open reading frame corresponds to the *SAC2* gene. The other reading frame just upstream of *SAC2* is the *MNT1* gene that encodes the α-1,2-mannosyltransferase, which had been sequenced previously by Hausler and Robbins (1992). This linkage with *MNT1* places *SAC2* on chromosome IV. By searching the GenBank and EMBL databases we were unable to detect any significant sequence similarities between *SAC2* and other proteins, indicating that *SAC2* encodes a novel protein.

To assess the *SAC2* null phenotypes, we created a *SAC2* gene disruption replacing most of the chromosomal *SAC2* gene with the *LEU2* marker gene. Only 16 N-terminal codons and about 100 codons at the C-terminus remained in the disrupted gene (Figure 1B). By transformation of the $Leu2^-$ diploid DBY1707, we produced a

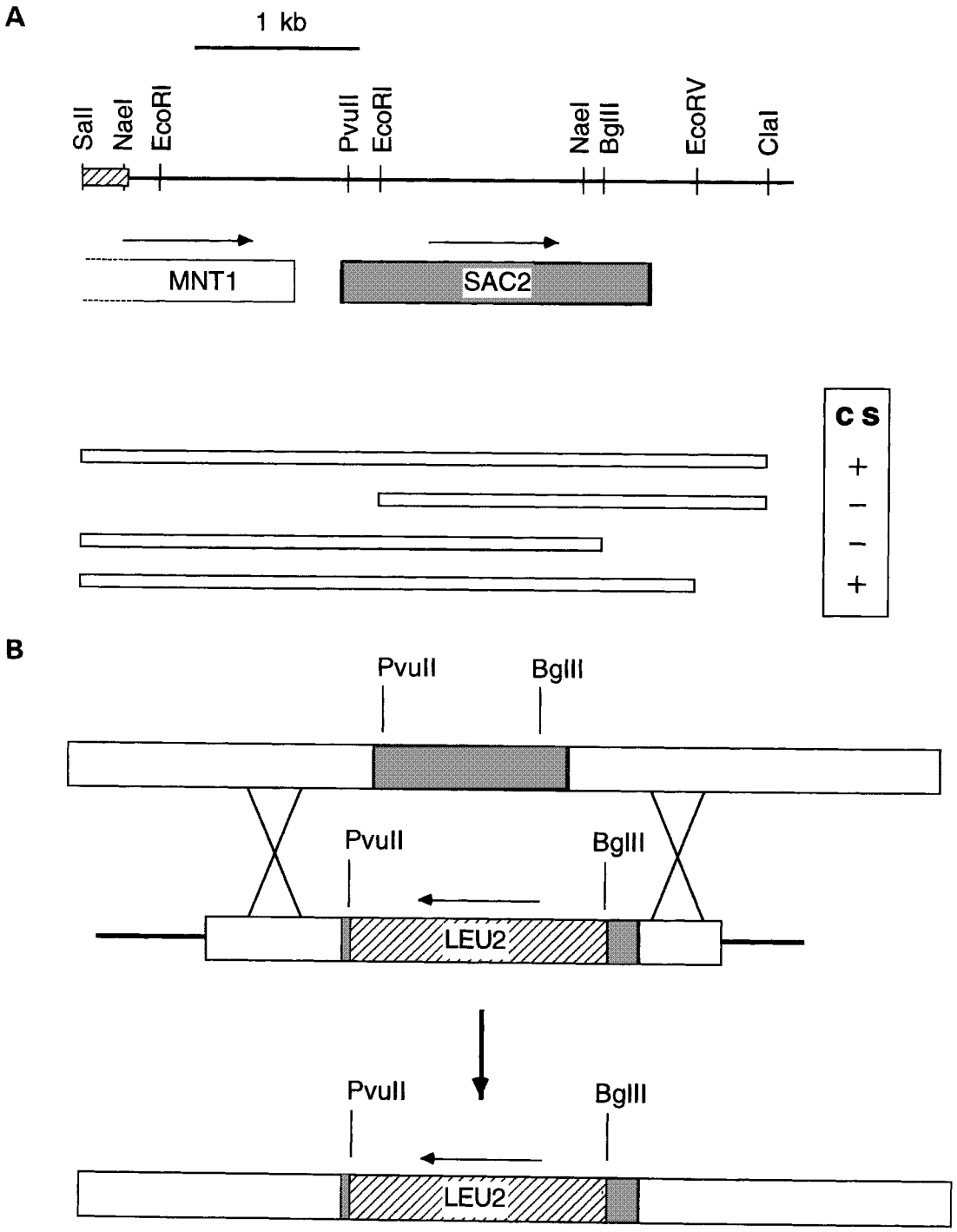


Figure 1. (A) Mapping of the *SAC2* gene by complementation. *SAC2* subclones were transformed into the *sac2-1* strain DBY1918. Transformants were tested for growth at 14°C (cs+ = growth, cs − = no growth). Indicated are the *SAC2* and *MNT1* open reading frames. The arrows point in the direction of transcription. (B) Disruption of the *SAC2* gene. Part of the *SAC2* gene (dark grey box) was replaced by the *LEU2* gene (hatched box) by homologous recombination between the chromosomal *SAC2* gene and the pRK23 fragment containing the truncated *SAC2* gene. The structure of the *SAC2* locus after the integration/deletion event is shown on the bottom of the diagram.

```
   1 GTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTG  90
  91 CATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGC 180
 181 GAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGG 270
 271 CGTAGAGGATCGACACTAAAACAACCATGGATTATATCACTCCATCTTTTGCTAACAAAGCTGGTAAGCCAAAAGCTTGTTACGTCACTT 360
 361 TGGTGAGAAACAAGGAGTTGAAAGGTTTGCTAAGCTCCATTAAATATGTGGAAAACAAAATTAACAAGAAATTCCCATATCCTTGGGTTT 450
 451 TCCTAAACGATGAACCTTTTACTGAAGAATTCAAGGAAGCAGTCACCAAAGCTGTTTCTTCCGAAGTTAAGTTTGGTATTTTGCCCAAGG 540
 541 AACATTGGTCATATCCTGAATGGATTAATCAAACCAAGGCTGCTGAAATTCGTGCAGATGCTGCCACCAAATACATATACGGTGGCTCCG 630
 631 AATCTTATAGACACATGTGTCGTTACCAATCTGGGTTTTTCTGGAGACATGAATTATTAGAAGAGTACGATTGGTACTGGCGTGTGGAAC 720
 721 CAGACATCAAGTTATACTGTGATATTAATTACGACGTTTTTAAGTGGATGCAAGAAAACGAAAAAGTTTACGGCTTTACCGTTTCTATTC 810
 811 ATGAATATGAAGTGACGATCCCAACACTATGGCAAACGTCCATGGATTTCATCAAAAAGAACCCCGAATACTTAGATGAAAACAACCTGA 900
 901 TGAGTTTTCTTTCGAACGATAATGGTAAAACATACAATCTGTGCCATTTCTGGTCAAACTTTGAAATTGCAAACTTGAATTTGTGGAGGT 990
 991 CACCAGCCTACAGAGAGTATTTTGACACTTTGGATCATCAAGGTGGATTTTTCTACGAAAGATGGGGCGATGCTCCCGTTCATTCTATTG 1080
1081 CTGCTGCTTTGTTTTTGCCAAAGGATAAAATCCATTATTTTTCAGACATTGGTTACCATCATCCACCTTATGATAACTGCCCATTGGACA 1170
1171 AGGAGGTCTATAACAGTAACAACTGTGAATGTGACCAAGGTAATGATTTCACTTTCCAAGGTTACTCTTGTGGTAAGGAATATTATGATG 1260
1261 CTCAAGGGTTGGTAAAGCCAAAAAACTGGAAAAAATTCCGTGAGTAGAAATCTTGGAACATACTGTTTCTTTGTTTTGACTTTATACTTT 1350
1351 CTATTTATATTTTATTTTTATAACTGGTTAAGTACACATAGGACTGCGTATCAAACATATAAGTGAGGCAATCCACATTTTTTTTAAAGA 1440
1441 TTCGAATATTTTTATTCTCATTAGCGTATTCCGAGAATAGTTCGAAAAAATATAAGGTATATCAAGAGTTTTTACAAGTGAGAGGAAAGA 1530
1531 GGAATAAGCTATAAGCAACAAAAGCGTAAAAAAATTAGCTGAAGACATAGAACTATGGATGTTCTCAAAGAGGTGTTGTCACTAGACCAA 1620
                                                    M  D  V  L  K  E  V  L  S  L  D  Q    12
1621 GATAAATTTGACCAGCTGAAGGAAACGAGCCGAGATAAAACAAATGAAACGGATGATCCTTTTGAAAACTATTTGAAGGATTGTAAATTT 1710
  13 D  K  F  D  Q  L  K  E  T  S  R  D  K  T  N  E  T  D  D  P  F  E  N  Y  L  K  D  C  K  F    42
1711 AAAGCGCCTTCAAACAAAGATCAGTCACCATTTGCTAAACTTAAATCATTACAGGAAACTCATTCTAACAATGAAGCGGCTATTAATATA 1800
  43 K  A  P  S  N  K  D  Q  S  P  F  A  K  L  K  S  L  Q  E  T  H  S  N  N  E  A  A  I  N  I    72
1801 ATTATTCCTCAATTGATTGATTACTTAACCGAATTCACTAATAGGTTATCAAATTACACACAAGATTTAGACTTCATTAAAAAAAAGTCC 1890
  73 I  I  P  Q  L  I  D  Y  L  T  E  F  T  N  R  L  S  N  Y  T  Q  D  L  D  F  I  K  K  K  S   102
1891 AATGAATTACAGTCATTGCTCGAATACAACTCCACTAAACTGGCACATATCTCTCCTATGGTTAATGATTTGATTCCTCCTGAACTC 1980
 103 N  E  L  Q  S  L  L  E  Y  N  S  T  K  L  A  H  I  S  P  M  V  N  D  L  M  I  P  P  E  L   132
1981 ATTGATGACATCATTAAAGGGAAGATCAATGAAAGCTGGCAGGATAATATAACATTCATAGCAGATAAAGAAGAAATTTATAACAAGTAT 2070
 133 I  D  D  I  I  K  G  K  I  N  E  S  W  Q  D  N  I  T  F  I  A  D  K  E  E  I  Y  N  K  Y   162
2071 AGGTCCAATAATCTCGATCAAGACAACAAGGACGCAGAAAATTCAGCAATGCTAGCACCAAAGGATTTTGATAAGTTATGTCAACTCCTG 2160
 163 R  S  N  N  L  D  Q  D  N  K  D  A  E  N  S  A  M  L  A  P  K  D  F  D  K  L  C  Q  L  L   192
2161 GACATCCTAAAAAATGTTATTCTAGAAAGGTCCGAAAAGACTTATTATTTCAAAATCAAAACTTTGAGGAGTCATAACCCAGTACCATCG 2250
 193 D  I  L  K  N  V  I  L  E  R  S  E  K  T  Y  Y  F  K  I  K  T  L  R  S  H  N  P  V  P  S   222
2251 CAAAGGATACAAAACAAATTATTAAAAGTTCAAAAAATTTTCCCCTTCATAAGAGATAATAATCTCTCCTTAGCCCTTGAGTTAAGACAG 2340
 223 Q  R  I  Q  N  K  L  L  K  V  Q  K  I  F  P  F  I  R  D  N  N  L  S  L  A  L  E  L  R  Q   252
2341 GCATATTGTTACACAATGAAATGGTATTATAGAGAATACTTTTCTAGATATATCAGGTCATTGACTATTTTGCAATTTCAACAAATCGAC 2430
 253 A  Y  C  Y  T  M  K  W  Y  Y  R  E  Y  F  S  R  Y  I  R  S  L  T  I  L  Q  F  Q  Q  I  D   282
2431 TCGCAATTTGCATTGGGTAATGGCCTTTCTACAACTTCAGTGAGTGGGTTTAACAATTCACCATCATTGTTTTTCTCAAATTATCTAACT 2520
 283 S  Q  F  A  L  G  N  G  L  S  T  T  S  V  S  G  F  N  N  S  P  S  L  F  F  S  N  Y  L  T   312
2521 ACATCCGCTTCAAATGCTTTCTATAATAAACTCCCTGTAACAGATGAGAAAATTGATAAATACTTTCAGATAAAGAAAAGATTGAACATT 2610
 313 T  S  A  S  N  A  F  Y  N  K  L  P  V  T  D  E  K  I  D  K  Y  F  Q  I  K  K  R  L  N  I   342
2611 TTAACACAAGAAGACAATACGGTAATGGTATCCCAAATTGCAGAAAATAACACAACGAAAAACTACATTGAAATTGGATTTAAAAATTTA 2700
 343 L  T  Q  E  D  N  T  V  M  V  S  Q  I  A  E  N  N  T  T  K  N  Y  I  E  I  G  F  K  N  L   372
2701 AACCTTGCAATTTTAGATAACTGTACGGTGGAGTACCATTTTTTAAAAGATTTCTTCGCTATGAATGGCGATAATTTTGAGGAAATTAAT 2790
 373 N  L  A  I  L  D  N  C  T  V  E  Y  H  F  L  K  D  F  F  A  M  N  G  D  N  F  E  E  I  N   402
2791 GGTTTATTGGAACAAATATTTCAACCAACTTTGATGAAGCCACAACATACACTCAACAACTGATCCAATATAATTATGACATTTTGGT 2880
 403 G  L  L  E  Q  I  F  Q  P  T  F  D  E  A  T  T  Y  T  Q  Q  L  I  Q  Y  N  Y  D  I  F  G   432
2881 GTATTAATAAGTATTCGTGTGGCCAATCAATTACAATTTGAATCAGAAAGGAGAGGAATACCGTCTATGTTTGATAGTTTCTTGAATGGT 2970
 433 V  L  I  S  I  R  V  A  N  Q  L  Q  F  E  S  E  R  R  G  I  P  S  M  F  D  S  F  L  N  G   462
2971 CAATTAATTCAATTATGGCCTCGATTTCAGCAATTGGTCGATTTTCAATGCGAGAGCTTACGAAAAGCGGCAATAACTACAAATGTGGCA 3060
 463 Q  L  I  Q  L  W  P  R  F  Q  Q  L  V  D  F  Q  C  E  S  L  R  K  A  A  I  T  T  N  V  A   492
3061 AAATATGCCGGCAACTCAAGCACATCCAATAGTAGCCCTTTGACCTCACCTCATGAGTTAACTGTACAGTTCGGTAAATTTTTATCAAGC 3150
 493 K  Y  A  G  N  S  S  T  S  N  S  S  P  L  T  S  P  H  E  L  T  V  Q  F  G  K  F  L  S  S   522
3151 TTCTTGACGTTGGCAATAACACATAAGCAGTCCATAGACGAAAGATCTGAACCCTTATACAATTCCATCATTAGATTAAGAAATGATTTC 3240
 523 F  L  T  L  A  I  T  H  K  Q  S  I  D  E  R  S  E  P  L  Y  N  S  I  I  R  L  R  N  D  F   552
3241 GAAACAGTCATGACAAAGTGCAGTAAAAAGACGAAATCACCAGAAAGATTTCTGGCTACAAATTACATGTATTTATACAATAACCTACAG 3330
 553 E  T  V  M  T  K  C  S  K  K  T  K  S  P  E  R  F  L  A  T  N  Y  M  Y  L  Y  N  N  L  Q   582
```

Figure 2.

```
3331  CAATTGCATCTACATTTAAATATAAATGACTCGGATGCACAAAACTACAATTTTGATTCTGCTGAAAATGTTGGTACGAAAGTTGCGAAT  3420
 583  Q  L  H  L  H  L  N  I  N  D  S  D  A  Q  N  Y  N  F  D  S  A  E  N  V  G  T  K  V  A  N   612

3421  GACGACGATAATGATTCAAGCGTACCACTAATAATCAGAGAGACCGAAAATCATTTCAAAACTTTAGTTGAAGCTTTCACCAGAAATTGA  3510
 613  D  D  D  N  D  S  S  V  P  L  I  I  R  E  T  E  N  H  F  K  T  L  V  E  A  F  T  R  N  *    642

3511  TGAATGTAACGATTGTATAAGACATGGAGCGTATAAAGTATATTTTTATGTAAAATGATCTTTATGATTTCTTATTTTGTGTGGAATATA  3600

3601  GTCAAATGTTTAACGACCTACCATTATATAGTCTGTCTCAATTAAAATCCTTCAGGAACACCTTTTGCAGGTCTTTGACTTAAATCAAGG  3690

3691  TAAATACCGCCGTTTTTGAACCCAAACCACTTGAATCTCACTTGATCGTCACTATCCTCATTTGTCGTTTCATCACGCTTGATATC  3776
```

Figure 2. *Continued*

Figure 2. The *SAC2* sequence.

strain (DBY5393), which is Leu$^+$ and heterozygous for this *SAC2* disruption allele. This diploid was sporulated and dissected. As was observed with the original *SAC2* alleles, haploid spores bearing the disruption proved to be unable to grow at 14°C. Cold-sensitivity segregated 2:2 in eight tetrads examined; as expected, all the cs spores were Leu$^+$ and all the cold-resistant spores were Leu$^-$.

The ability of the *SAC2* deletion to suppress the *act1-1* ts growth defect was tested by crossing DBY 5395 (*act1-1 TUB2::URA3*; the *URA3* gene is integrated near an intact *TUB2* gene) to DBY 5381 (*SAC2::LEU2*). The *URA3* gene was placed nearby to facilitate the scoring of suppression of the *act1-1* allele; *ACT1* and the *TUB2* gene are immediately adjacent to each other with only about 1 kb of DNA separating them. The diploid was sporulated and dissected. Out of 14 complete tetrads examined, 14 putative double mutant spores with the phenotype Ura3$^+$ Leu2$^+$ were obtained. Roughly half of these double mutant spores were ts$^+$, i.e. showed suppression of the *act1-1* ts defect. No reciprocal suppression was observed since all double mutant spores were cs, as expected from the presence of the *SAC2* deletion. Although suppression was always linked to the *SAC2* deletion (no ts$^+$ Ura3$^+$ Leu2$^-$ spores), the presence of the *SAC2* deletion alone was not sufficient for suppression. Since only 50% of all double mutant spores showed the ts$^+$ phenotype, another factor must be involved in suppression. It was noted before that suppression of *act1-1* by *sac2-1* is dependent on the strain background (Novick *et al.*, 1989).

In conclusion, we have characterized the *SAC2* gene, showing that it encodes a heretofore undescribed protein. The complete loss of this gene results in two phenotypes: cs growth and suppression, in some strain backgrounds, of the ts growth phenotype of the *act1-1* mutation.

REFERENCES

Adams, A. E. M., Botstein, D. and Drubin, D. G. (1989). A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation *Science* **243,** 231–233.

Adams, A. E. M. and Pringle, J. R. (1984). Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae. J. Cell Biol.* **98,** 934–945.

Gallwitz, D. and Seidel, R. (1980). Molecular cloning of the actin gene from yeast *Saccharomyces cerevisiae. Nucl. Acids Res.* **8,** 1043–1059.

Hausler, A. and Robbins, P. W. (1992). Glycosylation in *Saccharomyces cerevisiae*: cloning and characterization of an α-1,2-mannosyltransferase structural gene. *Glycobiol.* **2,** 77–84.

Ito, H., Fukuda, Y., Murada, K. and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153,** 163–168.

Kilmartin, J. V. and Adams, A. E. M. (1984). Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces. J. Cell Biol.* **98,** 922–933.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Messing, J. (1983). New M13 vectors for cloning. *Methods Enzymol.* **101,** 28–78.

Ng, R. and Abelson, J. (1980). Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* **77,** 3912–3916.

Novick, P., Osmond, B. C. and Botstein, D. (1989). Suppressors of yeast actin mutations. *Genetics* **121,** 659–674.

Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. and Fink, G. R. (1987). A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. *Gene* **60,** 237–243.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **85,** 4735–4739.

Sherman, F., Fink, G. and Lawrence, C. (1974). *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shortle, D., Haber, J. E. and Botstein, D. (1982). Lethal disruption of the yeast actin gene by integrative DNA transformation. *Science* **217,** 371–373.

Shortle, D., Novick, P. and Botstein, D. (1984). Construction and genetic characterization of temperature-sensitive mutant alleles of the yeast actin gene. *Proc. Natl. Acad. Sci. USA* **81,** 4889–4893.