MOLECULAR AND CELLULAR BIOLOGY, May 1990, p. 2308–2314
0270-7306/90/052308-07$02.00/0

Vol. 10, No. 5

Null Alleles of *SAC7* Suppress Temperature-Sensitive Actin Mutations in *Saccharomyces cerevisiae*

TERESA M. DUNN†* AND DAVID SHORTLE

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received 5 October 1989/Accepted 29 January 1990

Extragenic suppressors of a new temperature-sensitive mutation (*act1-4*) in the actin gene of *Saccharomyces cerevisiae* were isolated in an attempt to identify genes whose products interact directly with actin. One suppressor with a cold-sensitive growth phenotype defined the new gene, *SAC7*, which was mapped, cloned, sequenced, and disrupted. Genetic analysis of strains that are disrupted for *SAC7* demonstrated that the protein is required for normal growth and actin assembly at low temperatures. Surprisingly, null mutations in *SAC7* also suppressed the temperature-sensitive growth defect caused by the *act1-1* and *act1-4* mutations, whereas they were lethal in combination with the temperature-sensitive allele *act1-2*. These results support the notion that the *SAC7* gene product is involved in the normal assembly or function or both of actin.

Actin is a highly conserved protein which is present in all eucaryotic cells. The high conservation of its amino acid sequence implies a comparable conservation of actin functions common to all eucaryotic cells. Actin and its associated proteins are believed to function in maintenance of cell shape, organization of membrane proteins, and subcellular movements including organelle transport, cytoplasmic streaming, and cytokinesis (10).

Several temperature-sensitive mutations in the single yeast actin gene have been isolated and characterized (14, 22; unpublished data). The phenotypes associated with these mutations include disruption of the cell-cycle-dependent changes in the localization of actin in the cell, delocalized deposition of chitin on the cell surface, secretion defects and an accumulation of secretory vesicles, growth arrest at the unbudded stage of the cell cycle, and inability to grow on media with high osmolality. These observations support the assumption that the actin protein of yeasts has many of the same functions as that in higher eucaryotes.

The actin cytoskeleton in yeasts consists in part of a filamentous structure that undergoes dynamic reorganization during progression through the cell cycle (3, 6, 9). It is presumed that this reorganization is regulated by actin-associated proteins that function to control the assembly and disassembly of microfilaments. Several proteins that have the potential to influence the state of actin assembly have been identified by biochemical approaches (17). Recently, a genetic approach to identifying actin-associated proteins has also been undertaken (1, 2, 15). This strategy relies on the observation that in some instances, a conditional-lethal mutation in one protein can be suppressed by a compensating mutation in a protein that physically interacts with it (8). By concentrating on those suppressor mutations that have an associated conditional-lethal phenotype, the analysis is facilitated in several ways. For example, the suppressor mutations can be placed in complementation groups and the wild-type gene for the suppressor mutation can readily be cloned by complementation. More importantly, the associated phenotype can often be helpful in establishing that the suppressor mutation lies in a gene whose product plays a role in the same functional pathway, by showing that the conditionally lethal phenotype is similar to that of the original mutant (8, 11, 12).

Suppression analysis of *act1-1* haploid mutant cells has identified five genes that are capable of mutating to suppressors of the temperature-sensitive (Ts^-) phenotype and that simultaneously acquire a cold-sensitive (Cs^-) lethal phenotype (15). Three of these *SAC* (suppressor of actin) genes that have been extensively characterized exhibit properties that suggest they encode actin-associated proteins. All these *SAC* mutations are recessive for both their suppressing phenotype and their cold-sensitive phenotype, a surprising finding since suppressor mutations are generally dominant. When a homozygous *act1-1/act1-1* diploid was used for the selection of extragenic suppressors, one gene (*SAC6*) was identified that could mutate to dominant suppression of *act1-1*; this gene has subsequently been shown to encode a protein that is capable of binding actin filaments (1, 2).

One expected property of suppression involving direct interaction between a mutant protein and a suppressor protein is allele specificity. That is, since it should arise as a very specific compensating mutation in an actin-associated protein, the suppressor should suppress some but not all actin Ts^- mutations. We therefore undertook a suppression analysis of the *act1-4* Ts^- mutation with the expectation of identifying genes encoding a different set of actin-associated proteins than were selected by the *act1-1* mutation.

MATERIALS AND METHODS

Strains and media. All yeast strains used in this study are shown in Table 1 or were obtained from them by standard genetic crosses. Yeast media for growth and sporulation were prepared by the methods of Sherman et al. (21). Osmotic sensitivity was scored on plates containing ethylene glycol (14). Benomyl was added to autoclaved media to a final concentration of 50 μg/ml (24). *Escherichia coli* HB101 and DH5 were used, and media were prepared by the method of Schleif and Wensink (20).

Genetic techniques. Yeast mating, sporulation, and tetrad analysis were done by standard methods (22). Yeast transformation was done by the method of Ito et al. (7), except that 5 μg of sonicated salmon sperm DNA was routinely added to each transformation reaction.

Isolation of pseudorevertants. Spontaneous revertants of

* Corresponding author.
† Present address: Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

TABLE 1. Yeast strains used

Strain	Genotype
TDY2	*Matα ade2-101 ura3-52*
TDY42	*Matα ade2-101 ura3-52 act1-4*
B201	*Mata lys2 ura3-52 tub2-201*
TDY356	*Matα ade2-101 act1-4 sac7-1 ura3-52*
TDY202	*Matα SAC7::URA3 ade2-101 ura3-52*
TDY206	*Matα act1-1 ura3-52*
TDY221	*Matα sac7::LEU2 ade2-101 ura3-52 trp1 leu2*
TDY300	*Mata act1-2 leu2 his4-619 ura3-52*
DBY5224	*Mata ade8 arg4 leu2 ura3 trp4 aro1 rna3*
DBY5226	*Mata ade2 ura3 trp1 cdc9 rna11*

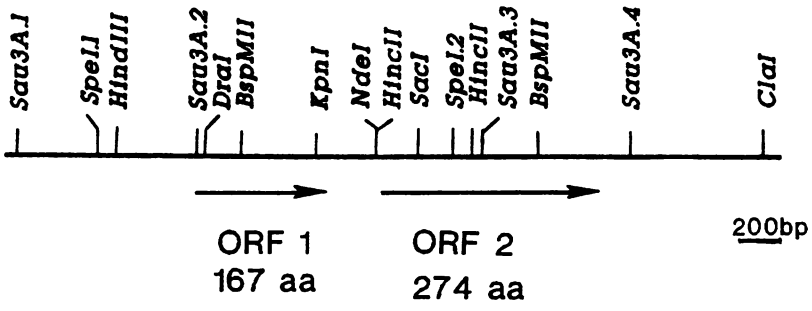
act1-4 were selected by streaking independent colonies of TDY42 (which were grown at 26°C) onto YPD (yeast extract-peptone-dextrose; 21) plates and incubating at 36°C for 3 days. The fastest-growing colony was picked for each independent TDY42 colony seeded and retested for growth at 36°C as well as at 26 and 12°C. Growth tests were done by streaking for single colonies and assessing colony size after 2 days (36°C), 3 days (26°C), or 12 days (12°C).

***SAC7* gene cloning.** The *SAC7* gene was cloned by complementation of the cold-sensitive phenotype of the *sac7-1* mutant. Strain TDY356 was transformed with plasmid DNA isolated from the yeast genomic library constructed by Rose et al. (18) in the centromere-containing yeast-*E. coli* shuttle vector YCp50. Transformants were selected on appropriately supplemented SD (synthetic dropout medium; 21) plates as uracil prototrophs at 12°C. Total DNA was isolated from six independent colonies (5) and used to transform *E. coli* DH5 to ampicillin resistance. Plasmid DNA was purified from *E. coli* and transformed back into the TDY356 strain, and uracil-prototrophic colonies selected at 26°C were screened for a Cs^+ phenotype.

Construction of integrating *SAC7* plasmid. One of the candidate *SAC7* clones in YCp50 was subcloned and a 2,700-base-pair (bp) *Hin*dIII-*Cla*I fragment was identified that complemented the *sac7-1* Cs^- mutant phenotype. This complementing fragment was ligated into the integrating vector YIp5 (4), and the resulting plasmid was linearized at the unique *Kpn*I site (located within the *Hin*dIII-*Cla*I fragment) and used for integrative transformation (16).

Subcloning and sequencing the *SAC7* gene. Further subcloning localized the *SAC7* gene to a 1,125-bp *Sau*3A fragment (*Sau*3A.2 to *Sau*3A.3; Fig. 1); this fragment and a larger 2,400-bp *Sau*3A fragment (*Sau*3A.1 to *Sau*3A.4, Fig. 1) were cloned into the *Bam*HI site of YCp50. To construct a number of insertion and deletion mutants for mapping the *SAC7* gene within the fragment as well as to facilitate subcloning into M13, the *Eco*RI-*Sph*I fragments of these plasmids were cloned into pUC18 and the *Kpn*I-*Eco*RI fragments were cloned into pUC19. Several M13 subclones were made and sequenced with the Sequenase kit (U.S. Biochemicals, Cleveland, Ohio). The insertion and deletion mutants that were used for mapping the *sac7-1*-complementing activity were generated by subcloning fragments of the *SAC7* gene into the polylinker cloning sites of the pRS314 vector (23).

Disruption of *SAC7* gene. The *SAC7*-disrupting allele



Complementation of the *sac7-1* phenotype

HindIII-ClaI	++++	*Spe1.1 -Spe1.2*	-
Sau3A.1-Sau3A.4	++++	*Spe1.1* - frameshift	++++
DraI-ClaI	++++	*SacI* - frameshift	-
Sau3A.2-Sau3A.3	+++	*Spe1.2* - frameshift	-
KpnI-ClaI	+	*HincII* deletion	-

FIG. 1. *SAC7* gene locus. Sequence analysis of the 1,125-bp *Sau*3A.2 to *Sau*3A.3 fragment identified the two open reading frames designated ORF1 and ORF2. Sequence analysis of larger subclones (*Sau*3A.1 to *Sau*3A.4) showed that ORF2 extends further. The ability of several restriction fragments and some frameshift and deletion mutations to complement the *sac7-1* Cs^- phenotype is indicated. The growth rate of a wild-type strain at 12°C is designated by ++++ and that of the *sac7-1* strain is designated by −. aa, Amino acids.

(*sac7*::*LEU2*$^+$) was generated by replacing the 480-bp *Hin*cII fragment of *SAC7* (Fig. 1) with a *Pvu*II fragment carrying the *LEU2*$^+$ gene. A *Bsp*MII fragment carrying the *sac7*::*LEU2*$^+$ allele was then used to transform strain TDY821 to leucine prototrophy (19). Diploid transformants were selected for DNA blot analysis, and genomic DNA was isolated and digested with *Hin*dIII and *Cla*I and then probed with the *Hin*dIII-*Cla*I *SAC7* fragment to identify *SAC7*/*sac7*::*LEU2*$^+$ heterozygotes.

Fluorescence microscopy. Diploid cells were grown, fixed, and stained for actin with rhodamine-conjugated phalloidin (Molecular Probes, Eugene, Oreg.) as previously described (3, 9).

RESULTS

Isolation of suppressor mutations. The temperature-sensitive lethal mutation *act1-4* (Glu-258 to Val) (unpublished observations) was used for the selection of suppressors. Yeast cells carrying the *act1-4* mutation grew normally at 26°C but became rapidly inviable upon shift to 36°C. One hundred independent unmutagenized colonies of strain TDY42 were plated at 36°C, and after 4 days, 31 independent large colonies were picked, purified, and retested for growth at 12, 26, and 36°C. To determine whether the suppressor mutation was linked to actin, each suppressor-containing isolate was backcrossed to B201 and analyzed by tetrad dissection to score linkage of the suppressor mutation to the benomyl-resistant allele of the β-tubulin gene, which is tightly linked (1 centimorgan) to the actin gene (24). For 16 of the isolates, the suppressed temperature-sensitive (Ts^{sup}) phenotype segregated away from the benomyl-sensitive phenotype in all 12 tetrads dissected. The suppressed phenotype of these Ts^{sup} mutants was therefore likely due to second-site mutations in the actin gene; in fact, for several of these candidates that conclusion was confirmed by cloning and sequencing the Ts^{sup} actin alleles. Eight of the Ts^{sup} isolates were not analyzed further owing to poor sporulation, low spore viability, or multiple mutations.

The remaining seven isolates contained suppressors that behaved as single mutations unlinked to the actin gene. Three of these seven extragenic suppressors resulted in cold-sensitive growth in the *act1-4* background; further analysis showed they were Cs$^-$ in the *ACT1*$^+$ background as well. Linkage analysis demonstrated that in each case, the associated Cs$^-$ phenotype was tightly linked to the suppressor mutation. In heterozygous diploids, these three suppressor mutations proved to be recessive for their associated Cs$^-$ phenotype. Results from linkage and complementation analysis of the suppressors were consistent with one of the Ts^{sup} mutations residing in one gene and the other two in a different gene. The gene for which one mutation was isolated was not analyzed further because, while it allows slow growth of the *act1-4* mutant strain at 36°C, it results in poor growth at all temperatures (12, 26, and 36°C).

We designated the gene for which two suppressor mutations were isolated as *SAC7*. The two *sac7* mutants behaved comparably with regard to suppression of *act1-4* and with regard to their growth rates at low temperature. Since no differences in phenotypes of the *sac7-1* and *sac7-2* isolates were observed, only *sac7-1* was used for the characterization of the *sac7* spontaneous mutation as described below. The degree of cold sensitivity (measured by growth rate relative to that of wild-type at a given temperature) of the *sac7-1* mutant varied with the actin allele. The *sac7-1 act1-4* double mutant was the most cold sensitive, the *sac7-1 act1-1* double mutant had an intermediate Cs$^-$ phenotype, and the *sac7-1 ACT1*$^+$ strain was the least cold sensitive.

TABLE 2. Dominant suppression of the *act1-4* and *act1-1* mutations by the *sac7-1* mutation

Strain	Relative growth rate[a]		
	ACT1$^+$/*ACT1*$^+$	*act1-4*/*act1-4*	*act1-1*/*act1-1*
SAC7$^+$/*SAC7*$^+$	++++	−	+
SAC7$^+$/*sac7-1*	++++	+	++
sac7-1/*sac7-1*	+++	++	+++

[a] The relative growth rate of these strains after 2 days of incubation at 36°C is scored, with ++++ indicating the wild-type growth rate.

Suppression of different actin alleles by *sac7-1*. In addition to suppressing the *act1-4* Ts$^-$ mutation, the *sac7-1* mutation also suppresses the Ts$^-$ phenotype of the *act1-1* mutant, which results from a Pro-32 to Leu substitution (14, 22). The *sac7-1* mutation was dominant for suppression of both the *act1-4* and the *act1-1* mutations (Table 2), a somewhat surprising result in that an earlier suppression analysis of the *act1-1* allele identified no dominant extragenic suppressors with associated Cs$^-$ phenotypes (15).

Interestingly, attempts to create a *sac7 act1-2* double mutant failed (the *act1-2* mutation arises from an Ala-58 to Thr change [22]). In other words, even though neither mutation alone is lethal, a cell carrying both mutations is inviable. This synthetic lethality in combination with *act1-2* is seen for the spontaneous *sac7* mutant allele as well as for a *sac7* null allele (the construction of the *sac7* null mutation is described below; because the cold-sensitive phenotype of *sac7* mutant cells takes 12 to 14 days to score, it was more convenient to generate the tetrad data presented in Table 3 by using the *sac7* null allele in which most of the *SAC7* gene was replaced by the *LEU2*$^+$ gene). As the data in Table 3 indicate, when the *SAC7*/*sac7*::*LEU2*$^+$ *ACT1*$^+$/*act1-2* doubly heterozygous strain (obtained by crossing TDY300 × TDY221) was sporulated and dissected, no viable *sac7 act1-2* (i.e., Leu$^+$ Ts$^-$) haploid products of meiosis were recovered. The pattern of spore viability was entirely consistent with this being due to inviability of the double mutant rather than to suppression of the *act1-2* Ts$^-$ phenotype by the *sac7* mutation. The *act1-2* mutation per se causes a higher frequency of spore inviability than is seen in the segregants that are *ACT1*$^+$ (note that for the six tetratype tetrads with two viable segregants, the presumed *act1-2* *SAC7*$^+$ haploids are inviable). Nonetheless, since all the four-spored tetrads that were recovered were parental ditype and since none of the 48 haploid segregants that could be deduced to be an *act1-2 sac7*::*LEU2*$^+$ double mutant were viable, it is apparent that the *act1-2* mutant cells require a functional *SAC7* gene for viability.

Isolation and nucleotide sequence of *SAC7* gene. The *SAC7*

TABLE 3. Inviability of the *act1-2 sac7* double mutant[a]

No. of viable spores	Parental ditype	Tetratype	Nonparental ditype
4	6	0	0
3	3	20	0
2	0	6	2[b]

[a] Tetrad analysis of a *SAC7*$^+$/*sac7*::*LEU2*$^+$ *ACT1*$^+$/*act1-2* diploid is presented.

[b] These two tetrads were deduced to be the nonparental ditype (i.e., 2 *sac7*:*LEU2*$^+$ *act1-2*:2 *SAC7*$^+$ *ACT1*$^+$), since the two viable haploid products of meiosis were *SAC7*$^+$ *ACT1*$^+$.

gene was cloned on the basis of its ability to complement the cold-sensitive growth phenotype of the *sac7-1* mutant strain. Five plasmids from a genomic library gave Cs^+ transformants of the *sac7-1* strain, with two of the five plasmids exhibiting significantly weaker complementation than the other three. Based on restriction mapping and Southern blot analysis, the weakly complementing class of genomic clones did not overlap the strongly complementing class. Furthermore, low-stringency DNA hybridization analysis failed to detect any homology between them (data not shown). Restriction fragments from each of the two classes of complementing clones were used to probe blots of yeast chromosomes separated by orthogonal field agarose gel electrophoresis. The DNA from the strongly complementing class hybridized to chromosome IV, while the DNA from the weakly complementing class hybridized to chromosome II or XIV (which were not well resolved). None of the previously identified *SAC* genes that have been mapped reside on either II or XIV.

The class of clones that strongly complemented the Cs^- growth defect (Fig. 1) was presumed to contain the bona fide *SAC7* gene. To confirm this, we marked the chromosomal sequence homologous to the complementing fragment with *URA3*$^+$ by integrative transformation of a complementing *URA3*$^+$ plasmid (16). Two haploid *URA3*$^+$ transformants of TDY2 were crossed to a *sac7-1* strain, and tetrad analysis confirmed that the *SAC7* locus had been marked since URA^+ and Cs^- segregated away from each other in 28 of 28 tetrads.

The entire DNA sequence of a 1,125-bp fragment (*Sau*3A.2 to *Sau*3A.3; Fig. 1) that was sufficient to complement the cold-sensitive phenotype of *sac7-1* mutant strain TDY356 was determined. Translation of the nucleotide sequence into amino acid codons indicated the presence of two long open reading frames which are designated ORF1 and ORF2 in Fig. 1. There are no consensus splice sites within this sequence. The ORF1 has no ATG within the first 66 codons; however, initiation from an ATG that lies about 200 bp into the cloned sequence would give rise to a 103-amino-acid protein. The ORF2 extends beyond the cloned fragment such that the potentially encoded protein would terminate downstream of the cloned fragment.

A number of additional subclones and deletion and insertion mutants (Fig. 1) were constructed to further localize the *SAC7* gene within the sequenced fragment. Any mutants that were missing or had altered sequence between the *Kpn*I site and the *Sau*3A.3 site at position 1125 were unable to complement. In contrast, constructs deleted or altered in sequence upstream of the *Kpn*I site were able to complement the *sac7-1* phenotype. On the basis of these results, it appears that ORF2 encodes the *SAC7* gene product.

When larger subclones were sequenced, it was found that ORF2 is capable of encoding a protein of 274 amino acids (Fig. 2). It is surprising that the amino-terminal 180 amino acids are capable of conferring complementation. This complementation by a gene fragment is not unique to the *sac7-1* mutation, since the 180-amino-acid fragment also complemented a *sac7* deletion mutant allele (see below). The sequence of the *SAC7* protein showed no significant homologies to sequences in the protein sequence data bases.

***SAC7* disruption.** Since the *sac7-1* mutant has a Cs^- lethal phenotype, it seemed likely that the *SAC7* gene would encode an essential function. To test this hypothesis, we constructed a null allele of *SAC7* (*sac7*::*LEU2*$^+$) by deleting a large portion of the *SAC7* sequence and replacing it with the *LEU2*$^+$ selectable marker (see Materials and Methods). Heterozygous *SAC7*/*sac7*::*LEU2*$^+$ strains were identified by Southern blot analysis and then sporulated and dissected. The somewhat surprising observation was made that *SAC7* is only essential for growth at low temperature, although the *sac7*::*LEU2*$^+$ mutant also grew significantly slower (60 to 70% as fast) than wild type at both 26 and 36°C.

A more surprising finding was that the *sac7*::*LEU2*$^+$ null allele conferred suppression of the Ts^- defects of the *act1-4* and *act1-1* mutants. Indeed, the spontaneously arising *sac7-1* mutation and the *sac7*::*LEU2*$^+$ disruption mutation behaved the same with regard to the Cs^- phenotype, suppression of the *act1-1* and *act1-4* Ts^- phenotypes, and inviability in combination with the *act1-2* mutation. Perhaps most surprising of all was the observation that the null allele of *SAC7* was partially dominant for suppression, indicating that a simple reduction in the level of *SAC7* product also results in suppression of the actin defect.

Two other phenotypes were observed for the *sac7*::*LEU2*$^+$ mutation. First, this mutation caused a recessive sporulation defect. Under sporulation conditions in which a homozygous *sac7*::*LEU2*$^+$ strain that is transformed with a YCp50-*SAC7*$^+$ plasmid yielded greater than 30% tetrads, the same strain transformed with the YCp50 vector alone yielded less than 1% tetrads. Second, the *sac7*::*LEU2*$^+$ mutant cells showed an aberrant pattern of actin assembly at their restrictive temperature. After 24 h at 12°C, they displayed an abnormally round cell shape and a pattern of actin staining that was very similar to that observed for the *act1-1* and *act1-4* mutants maintained at their restrictive temperature (14; unpublished observations). Instead of the normal cables of actin that are organized along the long axis of the mother cell (3, 9), only dots of actin-staining material were observed. This is shown for homozygous *sac7*::*LEU2*$^+$ diploid cells in Fig. 3B; similar results are obtained with *sac7-1* homozygous diploid cells (data not shown). Wild-type cells grown at 12°C showed cables of actin and the same mother-daughter asymmetry in the distribution of actin dots and cables that is characteristic of cells grown at higher temperatures before staining (Fig. 3A).

Mapping the *SAC7* gene. As described above, *SAC7* was shown to reside on chromosome IV by probing an OFAGE gel blot of separated yeast chromosomes. *SAC7* was more precisely mapped to the right arm of the chromosome between *ADE8* and *TRP4* by genetic linkage analysis with the chromosome IV mapping strains DBY5224 and DBY5226 (Table 4). No Cs^- mutants have previously been mapped to this region of the chromosome.

***SAC7* is distinct from *SAC1-SAC6*.** The suppression of the *act1-1* Ts^- phenotype by the *sac7* mutation raised the question whether this suppressing mutation is an allele of any of the previously identified *SAC* genes (1, 15). Several lines of evidence indicate that the *SAC7* gene is nonallelic with any of the *SAC1-SAC6* genes. For *SAC1*, *SAC2*, *SAC3*, and *SAC6*, the cloned wild-type genes failed to complement the *sac7-1* Cs^- phenotype, and no hybridization could be demonstrated between the cloned *SAC7* gene and any of the other cloned *SAC* genes (data not shown). The evidence against *SAC7* being allelic to *SAC4* or *SAC5* (which have not yet been cloned) is genetic complementation of the cold-sensitive phenotypes.

DISCUSSION

We isolated extragenic suppressors of an actin temperature-sensitive mutation in an attempt to identify genes that are involved in the organization and function of actin in vivo.

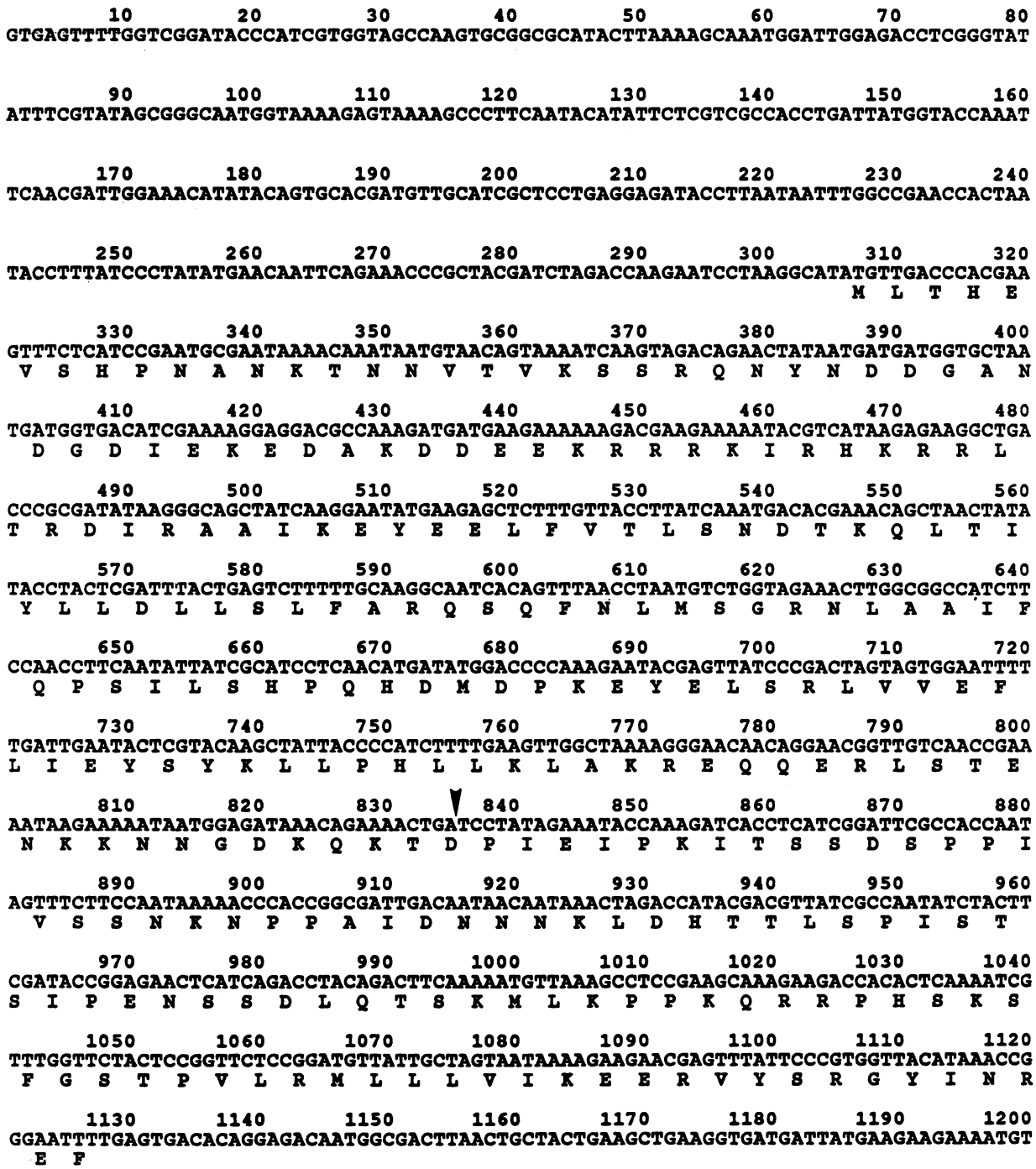


FIG. 2. Nucleotide sequence of the region containing the *SAC7* gene (GenBank accession number M32335). The predicted amino acid sequence of the 274-amino-acid open reading frame is shown. The position at which the gene can be truncated and still give rise to a complementing gene product is indicated by the arrowhead.

This approach has already been proved to be useful by Botstein and colleagues (1, 2, 15), who obtained suppressors of a different Ts^- actin allele (*act1-1*). Our assumption is that the broader the spectrum of actin mutations used in the suppression analysis, the greater the number of cytoskeletal components that are likely to be identified. Indeed, the suppressors that we isolated using the *act1-4* mutation were different from those isolated using the *act1-1* mutation. For example, many intragenic suppressors of *act1-4* were recovered, and one of the extragenic suppressors of *act1-4* with an associated Cs^- phenotype was dominant for suppression. Despite the fact that the null allele of *SAC7* suppresses *act1-1*, the *SAC7* gene was not identified in the suppression analysis of *act1-1*. This may reflect the fact that the *sac7* mutants are only significantly cold sensitive in the *act1-1* background when they are at or below 14°C.

We analyzed the extragenic suppressor *sac7-1* in some detail for several reasons. First, it has an associated Cs^- phenotype which suggested that it encodes an important if not essential function. The Cs^- property permitted the

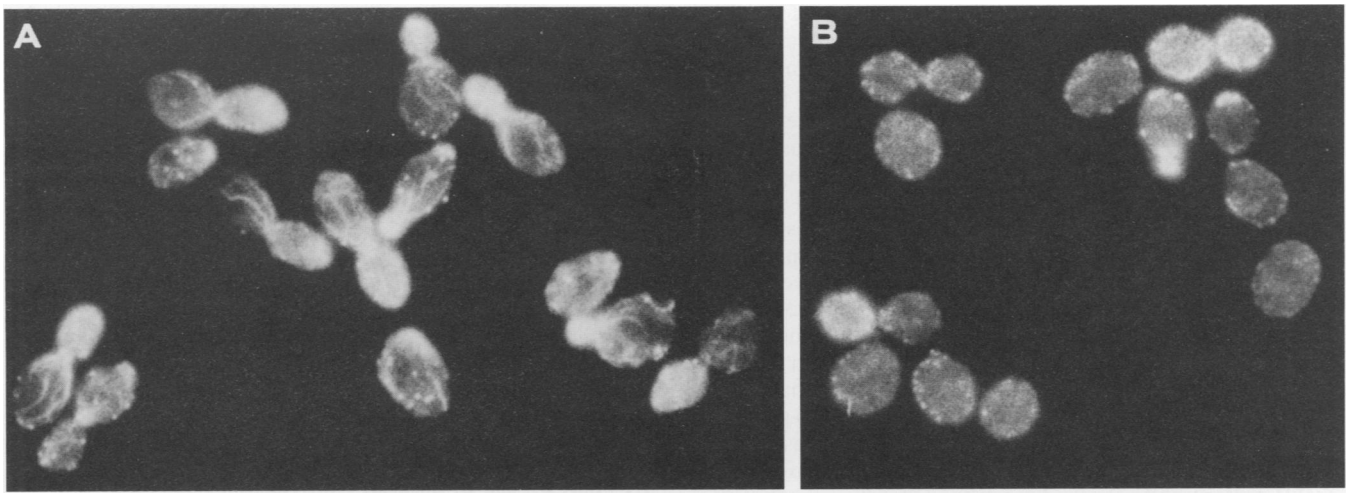


FIG. 3. Fluorescence localization of actin in yeast cells grown at 12°C. (A) Wild-type diploid cells. (B) Homozygous *sac7*::*LEU2*$^+$ cells.

phenotypic characterization of cells that are deficient in *SAC7* function and also facilitated cloning of the wild-type gene. Furthermore, the *sac7-1* suppressor has several properties that might be expected of an interactional suppressor. It is dominant for suppression, a property that could easily be imagined to be due to a gain of function such as tighter binding to the mutant actin protein. It displays a type of allele specificity since it suppresses *act1-1* and *act1-4* but is lethal in combination with *act1-2*. Finally, at their restrictive temperature, *sac7* mutant cells exhibit an aberrant assembly of actin, an observation which supports the idea that the *SAC7* gene product is involved in actin organization.

However, the prediction that a dominant suppressor displaying allele specificity and an associated conditional-lethal phenotype is likely to identify an essential gene that encodes an interactional suppressor is not realized for *SAC7*. First, the *SAC7* gene is only essential at low temperature. Second, the expectation that a dominant suppressor is likely to act by a gain of function is not met since the *SAC7* null allele also confers dominant suppression. Finally, the synthetic lethal phenotype of the *sac7-1 act1-2* double mutant is not due to a specific perturbation in the *SAC7* protein that results in an altered interaction between the two proteins, since the *SAC7* null allele is also lethal in combination with the *act1-2* mutation. Nonetheless, the aberrant actin assembly in the *sac7-1* and *sac7*::*LEU2*$^+$ mutants, along with the observation that the *SAC7* gene product is required for viability of the *act1-2* mutant, strongly indicates that the *SAC7* gene product may be required for normal actin assembly or function or both.

TABLE 4. Genetic mapping of *SAC7* on chromosome IV[a]

Genotype	Parental ditype	Tetratype	Nonparental ditype	Map distance (cM)[b]
SAC7/ade8	27	13	0	15
SAC7/trp4	32	8	0	10
ade8/trp4	24	16	0	23
rna3/SAC7	13	25	2	46
rna3/ade8	21	19	0	24
rna3/trp4	12	24	2	45

[a] Crosses were made between TDY202, in which the *SAC7* locus was marked by *URA3*$^+$, and DBY5224 and DBY5226.

[b] The map distance in centimorgans (cM) was calculated by the method of Mortimer and Schild (13).

The finding that the null allele confers suppression was unexpected since the *sac7-1* mutation is a dominant suppressor. However, it turns out that null alleles of *SAC7* are also dominant suppressors, since a diploid hemizygous for *SAC7* showed weak suppression of both the *act1-4* and *act1-1* Ts$^-$ phenotypes. Furthermore, in the cloning of *SAC7*, we identified a second gene that displays weak complementation of the *sac7-1* Cs$^-$ phenotype when present in two copies per cell. These observations suggest that the level of *SAC7* product (and perhaps of other functionally related proteins) in the cell plays an important role in the appropriate assembly of actin.

The *SAC7* gene has many properties in common with the *SAC1* gene identified in the suppressor analysis of the *act1-1* mutation (15). Mutant alleles of both genes result in a cold-sensitive phenotype, and analysis of null alleles demonstrated that each gene is only essential for growth at low temperature. Both mutants display an aberrant pattern of actin assembly at low temperature similar to that seen for the *act1-1* and *act1-4* mutants at their restrictive temperatures. Mutant alleles of both genes are lethal in combination with the *act1-2* mutation. Some putative null alleles of *SAC1* also confer suppression of *act1-1* (15). Both the *sac7* and the *sac1* mutations cause a recessive sporulation defect. The two mutants differ in that *sac1* is recessive for suppression, and the *sac1* mutant allele tested did not suppress *act1-4*. The similarities between the two genes raise interesting questions about their functional relatedness. We are currently generating strains that will allow us to determine the phenotype of a *sac1 sac7* double mutant.

The sequence of *SAC7* provides no obvious clues as to its function; no significant homologies to sequences in the protein databases were found. We did observe that the truncated gene which retains the potential to encode only the amino-terminal 180 amino acid residues (of 274) is able to fully complement the *sac7-1* and *sac7*::*LEU2*$^+$ alleles. There are no obvious differences between the truncated and the full-length gene in the ability to complement the Cs$^-$ phenotype, the sporulation defect, and the aberrant actin assembly.

How might the same protein that is essential for viability

at low temperatures provide for the suppression of the Ts^- lethal phenotype of the actin mutants at high temperatures? One possibility is that the assembly state of actin is controlled by somewhat different mechanisms over a range of temperatures, with *SAC7* playing an essential role only at low temperatures. One plausible model would assign *SAC7* a filament-severing activity. Perhaps at the high temperature at which the number of actin filaments in the Ts^- actin mutants is reduced, the absence of *SAC7* product serves to stabilize the remaining filaments and thereby suppress the Ts^- defect. This would seem inconsistent with the finding that the phenotype of *sac7* mutants at their restrictive temperature is also a diminished number of filaments; an explanation for this might be that nucleation of actin filament formation is inefficient at low temperature such that *SAC7* activity is required for severing existing filaments to generate nuclei for further filament growth. This idea is especially intriguing in view of the fact that fluorescence microscopy of *act1-2* mutant cells shows heavy bars of actin-staining material, indicating that the actin assembly defect is different from that for the *act1-1* and *act1-4* mutants (14). Perhaps *act1-2* mutant cells require *SAC7* protein to reduce the number of filaments even at the nominally permissive temperature.

We have recently expressed the *SAC7* protein in *E. coli* for the purpose of generating antibodies to *SAC7* that will allow us to address many of the questions raised about *SAC7* expression and function. In addition, the functional relationship between *SAC7* and the gene cloned on the basis of its ability to weakly complement the *SAC7* phenotype is also being pursued.

ACKNOWLEDGMENTS

We thank A. Adams, B. Osmond, P. Novick, and D. Botstein for helpful discussions, for providing data before publication, and for sending *SAC1-SAC6* strains and/or clones; P. Hieter for assistance in the OFAGE mapping and for providing the deletion allele of *leu2*; and M. Rose for the yeast genomic library.

Research was supported by Public Health Service grant GM34171 from the National Institutes of Health. T.D. was supported by a postdoctoral fellowship from the National Institutes of Health.

LITERATURE CITED

1. **Adams, A., and D. Botstein.** 1989. Dominant suppressors of yeast actin mutants that are reciprocally suppressed. Genetics **121**:675–683.
2. **Adams, A. E. M., D. Botstein, and D. G. Drubin.** 1989. A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. Science **243**:231–233.
3. **Adams, A. E. M., and J. R. Pringle.** 1984. Localization of actin and tubulin in wild type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98**:934–945.
4. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
5. **Holm, C., D. Meeks-Wagner, W. Fangman, and D. Botstein.** 1986. A rapid and efficient method for isolating DNA from yeast. Gene **42**:169–173.
6. **Huffaker, T. C., M. A. Hoyt, and D. Botstein.** 1987. Genetic analysis of the yeast cytoskeleton. Annu. Rev. Genet. **21**: 259–284.
7. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
8. **Jarvik, J., and D. Botstein.** 1975. Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. Proc. Natl. Acad. Sci. USA **72**:2738–2742.
9. **Kilmartin, J., and A. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98**:922–933.
10. **Korn, E. D.** 1978. Biochemistry of actomyosin-dependent cell motility (a review). Proc. Natl. Acad. Sci. USA **75**:588–599.
11. **Moir, D., and D. Botstein.** 1982. Determination of the order of gene function in the yeast nuclear division pathway using Cs and Ts mutants. Genetics **100**:565–577.
12. **Moir, D., S. E. Stewart, B. C. Osmond, and D. Botstein.** 1982. Cold-sensitive cell-division-cycle mutants of yeast: isolation, properties and pseudoreversion studies. Genetics **100**:547–563.
13. **Mortimer, R. K., and D. Schild.** 1980. The genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519.
14. **Novick, P., and D. Botstein.** 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40**:405–416.
15. **Novick, P., D. Botstein, and B. Osmond.** 1989. Suppressors of yeast actin mutations. Genetics **121**:659–674.
16. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78**:6354–6358.
17. **Pollard, T. D., and J. A. Cooper.** 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu. Rev. Biochem. **55**:987–1035.
18. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60**: 237–243.
19. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
20. **Schleif, R. F., and P. C. Wensink.** 1981. Practical methods in molecular biology. Springer-Verlag, New York.
21. **Sherman, F., G. Fink, and C. Lawrence.** 1974. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Shortle, D., P. Novick, and D. Botstein.** 1984. Construction and genetic characterization of temperature-sensitive alleles of the yeast actin gene. Proc. Natl. Acad. Sci. USA **81**:4889–4893.
23. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.
24. **Thomas, J. H., N. F. Neff, and D. Botstein.** 1985. Isolation and characterization of mutations in the β-tubulin gene of *Saccharomyces cerevisiae*. Genetics **112**:715–734.