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

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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 of both 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Several temperature-sensitive mutations in the single yeast actin gene have been isolated and characterized (14, 22; unpublished data).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 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-lact1-l diploid was used for the selection of extragenic suppressors, one gene (SAC6) was identified that could mutate to dominant suppression of act1-l; 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-l 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

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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) HindIII-Clal fragment was identified that complemented the sac7-1 Cs+ mutant phenotype. This complementing fragment was ligated into the integrating vector YLP5 (4), and the resulting plasmid was linearized at the unique KpnI site (located within the HindIII-Clal fragment) and used for integrative transformation (16).

**Subcloning and sequencing the SAC7 gene.** Further subcloning localized the SAC7 gene to a 1,125-bp Sau3A fragment (Sau3A.2 to Sau3A.3; Fig. 1); this fragment and a larger 2,400-bp Sau3A fragment (Sau3A.1 to Sau3A.4, Fig. 1) were cloned into the BamHI 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 EcoRI-SphI fragments of these plasmids were cloned into pUC18 and the KpnI-EcoRI 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

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**TABLE 1. Yeast strains used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>TDY2</td>
<td>Mata ade2-101 ura3-52</td>
</tr>
<tr>
<td>TDY42</td>
<td>Mata ade2-101 ura3-52 act1-4</td>
</tr>
<tr>
<td>B201</td>
<td>Mata lys2 ura3-52 tub2-201</td>
</tr>
<tr>
<td>TDDY356</td>
<td>Mata ade2-101 act1-4 sac7-1 ura3-52</td>
</tr>
<tr>
<td>TDDY202</td>
<td>Mata SAC7::URA3 ade2-101 ura3-52</td>
</tr>
<tr>
<td>TDDY206</td>
<td>Mata act1-1 ura3-52</td>
</tr>
<tr>
<td>TDDY221</td>
<td>Mata sac7::LEU2 ade2-101 ura3-52 trp1 leu2</td>
</tr>
<tr>
<td>TDDY300</td>
<td>Mata act1-2 leu2 his4-619 ura3-52</td>
</tr>
<tr>
<td>DBY5224</td>
<td>Mata ade8 arg4 leu2 ura3 trp4 aro1 rna3</td>
</tr>
<tr>
<td>DBY5226</td>
<td>Mata ade2 ura3 trp1 cdc9 rna11</td>
</tr>
</tbody>
</table>

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**FIG. 1. SAC7 gene locus.** Sequence analysis of the 1,125-bp Sau3A.2 to Sau3A.3 fragment identified the two open reading frames designated ORF1 and ORF2. Sequence analysis of larger subclones (Sau3A.1 to Sau3A.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 HincII fragment of SAC7 (Fig. 1) with a PvuII fragment carrying the LEU2* gene. A BspMII 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 HindIII and Clal and then probed with the HindIII-Clal 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 actl-4 (Glu-258 to Val) (unpublished observations) was used for the selection of suppressors. Yeast cells carrying the actl-4 mutation grew normally at 26°C but became rapidly inviable upon shift to 36°C. One hundred independent unmutilagenized 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 μ-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 phenotype in the actl-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 actl-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 actl-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 actl-4 double mutant was the most cold sensitive, the sac7-1 actl-1 double mutant had an intermediate Cs− phenotype, and the sac7-1 ACT1+ strain was the least cold sensitive.

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

Interestingly, attempts to create a sac7 actl-2 double mutant failed (the actl-2 mutation arises from an Ala-58 to Thr change [22]). In other words, even though mutation alone is lethal, a cell carrying both mutations is inviable. This synthetic lethality in combination with actl-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+actl-2 double heterozygous strain (obtained by crossing TDY300 × TDY221) was sporulated and dissected, no viable sac7 actl-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 actl-2 Ts− phenotype by the sac7 mutation. The actl-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 tetraspore tetrads with two viable segregants, the presumed actl-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 actl-2 sac7::LEU2* double mutant was viable, it is apparent that the actl-2 mutant cells require a functional SAC7 gene for viability.

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

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relative growth rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT1+ACT1+</td>
<td>actl-4/actl-4</td>
</tr>
<tr>
<td>SAC7*/SAC7*</td>
<td>++++</td>
</tr>
<tr>
<td>SAC7*/sac7-1</td>
<td>++++</td>
</tr>
<tr>
<td>sac7-1/sac7-1</td>
<td>+++</td>
</tr>
</tbody>
</table>

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

**TABLE 3. Inviability of the actl-2 sac7* double mutant**

<table>
<thead>
<tr>
<th>No. of viable spores</th>
<th>Parental ditype</th>
<th>Tetratype</th>
<th>Nonparental ditype</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>6</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Tetrad analysis of a SAC7+/sac7::LEU2* ACT1+actl-2 diploid is presented.
* These two tetrads were deduced to be the nonparental ditype (i.e., 2 sac7::LEU2* actl-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 URA3+ and Cs" segregated away from each other in 28 of 28 tetrads.

The entire DNA sequence of a 1,125-bp fragment (Sau3A.2 to Sau3A.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 AGT within the first 66 codons; however, initiation from an AGT 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 be translated in a unique reading frame.

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 KpnI site and the Sau3A.3 site at position 1125 were unable to complement. In contrast, constructs deleted or altered in sequence upstream of the KpnI 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 actl-1 and actl-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 actl-1 and actl-4 Ts" phenotypes, and inviability in combination with the actl-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 actl-1 and actl-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 actl-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.
The nucleotide sequence of the region containing the SAC7 gene (GenBank accession number M32335). The predicted amino acid sequence of the 274-aa 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 Tsz- actin allele (actl-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 actl-4 mutation were different from those isolated using the actl-1 mutation. For example, many intragenic suppressors of actl-4 were recovered, and one of the extragenic suppressors of actl-4 with an associated Cs\(^+\) phenotype was dominant for suppression. Despite the fact that the null allele of SAC7 suppresses actl-1, the SAC7 gene was not identified in the suppression analysis of actl-1. This may reflect the fact that the sac7 mutants are only significantly cold sensitive in the actl-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
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 actl-1 and actl-4 but is lethal in combination with actl-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 actl-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 actl-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 actl-2 mutant, strongly indicates that the SAC7 gene product may be required for normal actin assembly or function or both.

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 actl-4 and actl-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 SAC7 gene identified in the suppressor analysis of the actl-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 actl-1 and actl-4 mutants at their restrictive temperatures. Mutant alleles of both genes are lethal in combination with the actl-2 mutation. Some putative null alleles of SAC7 also confer suppression of actl-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 actl-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. 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.

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LITERATURE CITED