Dominant Suppressors of Yeast Actin Mutations That Are Reciprocally Suppressed

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Manuscript received August 11, 1988
Accepted for publication December 27, 1988

ABSTRACT

A gene whose product is likely to interact with yeast actin was identified by the isolation of pseudorevertants carrying dominant suppressors of the temperature-sensitive (Ts) *act1-1* mutation. Of 30 independent revertants analyzed, 29 were found to carry extragenic suppressor mutations and of these, 24/24 tested were found to be linked to each other. This linkage group identifies a new gene *SAC6*, whose product, by several genetic criteria, is likely to interact intimately with actin. First, although *act1-1 sac6* strains are temperature-independent (Ts^+), 4/17 *sac6* mutant alleles tested are Ts in an $ACT1^+$ background. Moreover, four Ts^+ pseudorevertants of these $ACT1^+$ *sac6* mutants carry suppressor mutations in *ACT1*; significantly, three of these are again Ts in a $SAC6^+$ background, and are most likely new *act1* mutant alleles. Thus, mutations in *ACT1* and *SAC6* can suppress each other's defects. Second, *sac6* mutations can suppress the Ts defects of the *act1-1* and *act1-2*, but not *act1-4*, mutations. This allele specificity indicates the *sac6* mutations do not suppress by simply bypassing the function of actin at high temperature. Third, *act1-4 sac6* strains have a growth defect greater than that due to either of the single mutations alone, again suggesting an interaction between the two proteins. The mutant *sac6* gene was cloned on the basis of dominant suppression from an *act1-1 sac6* mutant library, and was then mapped to chromosome *IV*, less than 2 cM from *ARO1*.

UNTIL recently, information concerning the eukaryotic cytoskeleton had been obtained almost exclusively from biochemical, morphological, and inhibitor studies of higher cells. It has become clear that these cells possess at least three systems of cytoskeletal elements, based on a filament composed of actin, tubulin, or intermediate-filament proteins, and that each system is complex, comprised of a large number of interacting components.

The beginning of a genetic analysis of the yeast *Saccharomyces cerevisiae* actin cytoskeleton by means of pseudoreversion is presented in NOVICK, OSMOND and BOTSTEIN (1989). Five genes, *SAC1-SAC5*, were identified that could give rise to suppressors of mutations in *ACT1*, the single essential gene that encodes actin in this yeast (GALLWITZ and SEIDEL 1980; GALLWITZ and SURES 1980; NG and ABELSON 1980; SHORTLE, HABER and BOTSTEIN 1982). The phenotypes of temperature-sensitive *act1* mutations (SHORTLE, NOVICK and BOTSTEIN 1984; NOVICK and BOTSTEIN 1985) provided support for the idea, originally derived from fluorescence-localization studies (ADAMS and PRINGLE 1984; KILMARTIN and ADAMS 1984) that actin is involved in the polarization of growth and secretion. The similar phenotypes of the *sac* mutations in NOVICK, OSMOND and BOTSTEIN (1989) lend support to the idea that the *SAC* genes might encode proteins that interact with actin in the yeast cytoskeleton.

To isolate the *sac* mutants, strains carrying single mutations (Sup/Cs) that had acquired a cold-sensitive growth phenotype as well as the ability to suppress a temperature-sensitive defect in the actin gene were sought [NOVICK, OSMOND and BOTSTEIN (1989); see JARVIK and BOTSTEIN (1975) for the rationale]. Quite unexpectedly, all of these suppressor mutations turned out to be *recessive*, not only for their Cs phenotype, but also for their suppression phenotype. In the only previous studies of such pseudo-revertants in yeast (MOIR *et al.* 1982), most of the mutations were, as might be expected, *dominant* for their suppression phenotype. We therefore decided to extend the analysis of suppressors of *act1* mutations in yeast by looking specifically for *dominant* suppressors. We report here the identification of a new gene, *SAC6*, that gives rise to dominant suppressor mutations that themselves are suppressed by *act1* alleles, suggesting that the *SAC6* product is likely to interact closely with actin.

MATERIALS AND METHODS

Strains and media: The yeast strains used as parents and/or testers in this study are listed in Table 1. All other strains were derived from these using standard genetic methods (SHERMAN, FINK and LAWRENCE 1974), and are described in the text, Table 3, or Table 5. Media for yeast growth and sporulation were as described by SHERMAN, FINK and LAWRENCE (1974), except benomyl plates were as described by SCHATZ, SOLOMON and BOTSTEIN (1986).

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TABLE 1

Yeast strains used in this study[a]

Strain	Genotype[b]
DBY845	*MATα his4-619 cdc2-1*
DBY846	*MAT***a** *ade2-1 cdc2-1*
DBY848	*MATα his4-619 cdc9-1*
DBY849	*MAT***a** *ade2-1 cdc9-1*
DBY877[c]	*MATα his4-619 MOX1*
DBY1103	*MATα his4 cdc46*
DBY1104	*MAT***a** *ade2 cdc46*
DBY1827	*MATα his3-200 leu2-3,112 ura3-52*
DBY1884	*MATα act1-1 sac1-6 his4-619*
DBY1915	*MAT***a** *act1-1 sac1-15 ura3-52*
DBY1916	*MAT***a** *act1-1 sac2-1 MOX1 ura3-52*
DBY1920	*MATα act1-1 sac2-1 MOX1 his4-619*
DBY1962	*MATα act1-1 sac3-2 his4-619*
DBY1973	*MAT***a** *act1-1 sac4-2 MOX1 ura3-52*
DBY1974	*MATα act1-1 sac4-2 MOX1 his4-619*
DBY1985	*MAT***a** *act1-1 sac5-1 MOX1 ura3-52*
DBY1986	*MATα act1-1 sac5-1 MOX1 his4-619*
DBY1991	*MATα act1-2 MOX1 his4-619*
DBY1993	*MATα act1-2 MOX1 ura3-52*
DBY1995	*MATα act1-1 his4-619 MOX1*
DBY1997	*MAT***a** *act1-1 lys2-803*
DBY1998	*MATα act1-1 mox1-1 his4-619 can1*
DBY2001	*MAT***a** *act1-1 tub2-201 ura3-52*
DBY2017	*MAT***a** *act1-1 mox1-1 ura3-52 can1*
DBY2057[c]	*MAT***a** *ura3-52*
DBY2059[c]	*MATα leu2-3,112*
DBY2060[c]	*MAT***a** *leu2-3,112*
DBY2063[c]	*MAT***a** *ura3-52 leu2-3,112*
DBY2065[c]	*MAT***a** *lys2-801*
DBY2066[c]	*MATα lys2-801 his4-619*
DBY2326	*MATα act1-4 ura3-52 ade2-101*
DBY5217[d]	*MAT***a**/*MATα act1-1/act1-1 tub2-201/+ ura3-52/+ his4-619/+*
DBY5218[e]	*MAT***a** *act1-1 tub2-201 sac6-6 ura3-52*
DBY5220[f]	*MAT***a** *sac6-2 ura3-52*
DBY5221[f]	*MATα sac6-6 ura3-52*
DBY5222[f]	*MAT***a** *sac6-7 ura3-52*
DBY5224[g]	*MAT***a** *ade8 arg4 leu2 ura3 trp4 aro1C rna3 gcn4? pet2?*
DBY5227	*MAT***a** *ade8 ade2 gal2 lys7 aro1B hom2 trp4 ura3*
DBY5228[h]	*MATα act1-1 aro1B hom2 ura3*
DBY5229[e]	*MAT***a** *act1-1 tub2-201 sac6-6 his4-619*
DBY5230	*MAT***a** *act1-2 ura3-52*
DBY5231[i]	*MAT***a** *act1-4 ura3-52 leu2-3,112*
DBY5232[i]	*MATα act1-4 his4-619*
DBY5234	*MAT***a** *trp4 ura3 ade8 rna3 leu2 gcn4? met pet2*
DBY5235	*MATα aro1C ura3 arg4 rna3 leu2 gcn4?*

All strains were made in our laboratory except for DBY2326, obtained from T. DUNN and D. SHORTLE, and DBY5227, DBY5234 and DBY5235, which were kindly provided by G. FINK.

[a] The strains listed are those used as parent and/or tester strains. All other strains were derived from these by standard genetic methods, and are described in the text, Table 3, or Table 5.

[b] Genotypes at the *MOX1* locus are unknown, unless stated. Otherwise, full genotypes are listed except as noted.

[c] Except for the markers shown, these strains are essentially isogenic, as they are segregants from the 10th backcross to DBY877 (B. OSMOND, personal communication). They were therefore used interchangeably as wild-type strains.

[d] A diploid obtained by crossing DBY1995 × DBY2001.

[e] Haploid segregants from mutant 6 (Tables 2 and 3).

[f] Segregants from crosses shown in Table 2.

[g] A segregant from the cross of DBY5234 × DBY5235.

[h] A segregant from DBY5227 × DBY1995. Only the relevant genotype is shown.

[i] Segregants from two successive backcrosses of DBY2326 to DBY2063 and DBY877.

For all bacterial manipulations, *Escherichia coli* strain HB101 (BOYER and ROULLAND-DUSSOIX 1969) was used, and media were as described by DAVIS, BOTSTEIN and ROTH (1980).

Genetic techniques: Yeast mating, sporulation, and tetrad analysis were performed as described by SHERMAN, FINK and LAWRENCE (1974). Growth on plates was scored by spotting suspensions of cells in water onto plates using a 32-point inoculator. In all cases where dominance was tested, genotypes of the diploids were confirmed by tetrad analysis.

Isolation of revertants: Spontaneous, temperature-resistant (Ts^+) revertants were isolated by seeding YEPD plates [250–300 for DBY5217; 10 for each *sac6* mutant strain (see RESULTS)] with about 10^6 cells per plate, and incubating for 3–5 days at restrictive temperature (37°). To ensure that each revertant isolated was independent, cells from a single colony grown at permissive temperature (26°) were used to seed each plate, and only one revertant was picked per plate. Revertants were purified by streaking for single colonies on YEPD medium at 37°. During the course of the analysis, a large number of revertants were discarded because of poor sporulation, low spore viability, or failure to yield Ts^+ haploid segregants (see RESULTS). It is worth noting that almost all revertants retained were among those that grew most vigorously at 36°.

Molecular cloning of the *sac6* gene: Yeast genomic DNA was isolated from strain DBY5218 (*act1-1 sac6-6*) by the method of CRYER, ECCLESSHALL, and MARMUR (1975). A partial *Sau3A* digest was used to generate fragments, which were then separated on a 10–40% sucrose gradient. Fractions containing DNA 10–15 kb were pooled, and fragments were inserted into YCp50 [C. MANN, unpublished data; see MA *et al.* (1987) for a restriction map] that had been digested with *Bam*HI and treated with calf intestinal alkaline phosphatase (Boehringer Mannheim). The ligation mixture was used to transform *E. coli* strain HB101, and approximately 33,000 transformant colonies were pooled. Plasmid DNA was isolated without further amplification, and was used to transform strain DBY2001 (*act1-1*) by the lithium acetate method of ITO *et al.* (1983) as modified by KUO and CAMPBELL (1983) with sonicated salmon sperm DNA (Sigma) as carrier. Cells were plated on minimal plates lacking uracil (to select Ura^+) at 26° for 22 hr, and were then shifted to 37° (to select suppression) for 3 days. From an estimated 7000 transformants, only one large Ts^+ colony was obtained. This was purified by streaking for single colonies on selective medium at 26°. Plasmid (pRB1275) was isolated from this transformant by the method of HOFFMAN and WINSTON (1987) and was amplified in *E. coli* strain HB101.

A 4.1-kb *Eco*RI fragment containing part of the wild-type *SAC6* gene was isolated by D. DRUBIN (ADAMS, BOTSTEIN and DRUBIN 1989). It was inserted into the *Eco*RI site of YIp5 (which contains the selectable yeast *URA3* gene and no yeast origin of replication (BOTSTEIN *et al.* 1979)] to yield plasmid pRB1276.

RESULTS

Isolation of revertants carrying dominant suppressor mutations: In order to isolate pseudorevertants carrying dominant extragenic suppressors of the *act1-1* mutation, an *act1-1/act1-1* homozygous mutant diploid strain (DBY5217) was used. Analysis of the revertants (see below) was facilitated by the fact that DBY5217 is heterozygous for the *tub2-201* mutation, which is tightly linked to *ACT1*, and so serves as a

marker for this locus; *tub2-201* confers on the cells benomyl resistance, and has no temperature conditionality associated with it.

As described in MATERIALS AND METHODS, 111 spontaneous, independent Ts^+ revertants of strain DBY5217 were isolated. These were sporulated, and the segregation of temperature and benomyl resistance was followed in tetrad analysis. Of the 111 revertants, 77 sporulated poorly, showed low spore viability or failed to yield Ts^+ segregants; these, and four others, were not analyzed further. In 27 of the remaining 30 revertants, temperature resistance segregated 2:2, indicating a single, nuclear, mutation. In 26 of these revertants (mutants 1–26), the mutations were unlinked to *tub2-201*, and thus are extragenic suppressors (*i.e.*, "*sac*" mutants) (NOVICK, OSMOND and BOTSTEIN 1989). In the other revertant, the mutation was tightly linked to *tub2-201* (16PD:0T:0NPD), and so is either intragenic, or a tightly linked extragenic suppressor. The remaining three revertants (mutants 28–30) yielded an excess of Ts^+ segregants. However, when benomyl-resistant, Ts^+ haploids (*act1-1 tub2-201 sac*) from these were crossed to strain DBY1995 (*act1-1 TUB2*$^+$ *SAC*$^+$), temperature resistance was found to segregate 2:2 and to be unlinked to benomyl resistance. Thus, these revertants also carry extragenic suppressor mutations, and either underwent mitotic recombination or gene conversion, or accumulated additional suppressors during vegetative growth.

***act1-1* and *sac* mutations show reciprocal suppression:** Suppressor mutations have sometimes been observed to cause concomitant recessive temperature-conditional lethality, a result that has greatly assisted in the analysis of these suppressors (*e.g.*, JARVIK and BOTSTEIN 1975; MORRIS, LAI and OAKLEY 1979; MOIR *et al.* 1982; NOVICK, OSMOND and BOTSTEIN 1989). Consequently, haploid Ts^+ segregants from the 30 diploid revertants (above) were tested for cold sensitivity. In each case, the segregants were not cold sensitive. However, when benomyl-resistant, Ts^+ (*tub2-201 act1-1 sac*) segregants from 17 revertants were crossed to wild type, in four cases (mutants 2, 6, 7 and 19) two temperature-sensitive mutations were found to segregate (Table 2). These mutations are distinguishable phenotypically as one of them causes a growth defect at higher temperatures only (and is said to be Ts Cs^+), whereas the other results in poor growth at all temperatures (and is said to be Ts Cs). The former is the *act1-1* mutation as it is linked to benomyl resistance; the latter is manifested only in benomyl-sensitive (*ACT1*$^+$) segregants, and in further crosses to wild type, showed 2:2 segregation (not shown). The tetrad data shown in Table 2 indicate that in each of the four cases, the two Ts mutations are unlinked. We conclude that strains carrying either of them are Ts, whereas strains carrying *both* are Ts^+.

TABLE 2

Tetrad analysis of crosses between *act1-1 tub2-201 sac* and *ACT1*$^+$ *TUB2*$^+$ *SAC*$^+$ strains[a]

	No. of tetrads[b] Ts$^+$:Ts					Segregants[c]		
Mutant	4:0	3:1	2:2	1:3	0:4	Ts Cs$^+$ Ben^r:Ben^s	Ts Cs Ben^r:Ben^s	Inferred No. of Ts mutations segregating[b]
2	3	2*	14	0	1	18:0	0:16	2
3	1	4	1	0	0	6:0	0:0	1
4	1	4	1	0	0	6:0	0:0	1
6	5	0	10	1*	4	17:1$^+$	0:20	2
7	4	0	18	1*	1	19:2$^+$	3:19$^+$	2
8	2	2	2	0	0	n.d.	n.d.	1
10	1	4	1	0	0	6:0	0:0	1
11	1	3	2	0	0	7:0	0:0	1
14	0	6	0	0	0	6:0	0:0	1
15	4	13	1	0	0	14:1$^+$	0:0	1
16	3	2	0	0	0	2:0	0:0	1
17	0	3	2	0	0	6:1$^+$	0:0	1
18	1	5	0	0	0	5:0	0:0	1
19	4	2*	14	0	3	20:0	0:22	2
20	1	4	0	0	0	4:1$^+$	0:0	1
22	2	4	0	0	0	4:0	0:0	1
24	1	4	1	0	0	6:0	0:0	1

[a] Crosses were made between *act1-1 tub2-201 sac* segregants (derived from the diploid mutants listed) and wild-type strain DBY877, DBY2057, DBY2065, or DBY2066.

[b] The presence of tetrads with $0^+:4^-$, and the lack of tetrads with $3^+:1^-$, indicates that two Ts mutations are segregating. When two Ts mutations segregate, $4^+:0^-$ = PD; $2^+:2^-$ = TT; $0^+:4^-$ = NPD, and when only one Ts mutation segregates, $4^+:0^-$ = PD; $3^+:1^-$ = TT; $2^+:2^-$ = NPD. The odd tetrads, indicated by (*), are presumably false tetrads or the result of gene-conversion events.

[c] Most of the Ts Cs^+ segregants are Ben^r, and thus carry the *act1-1* mutation. In four cases (mutants 2, 6, 7 and 19), Ts Cs segregants also arise; as most of these are Ben^s, they carry the *ACT1*$^+$ allele. The few exceptions [marked by (†)] are presumably due to reciprocal recombination or gene-conversion events in the *ACT1-TUB2* interval.

Thus, each mutation appears to suppress the other's defect (Figure 1).

Confirmation that the *act1-1* mutation was able to suppress the growth defect of the Ts Cs *sac* mutations was obtained by crossing *tub2-201 act1-1 sac* strains (derived from mutants 6, 7 or 19) to *TUB2*$^+$ *ACT1*$^+$ *sac* strains (in each case, the diploids formed were homoallelic at the *sac* locus). When these diploids were sporulated and tetrads dissected, temperature resistance was found to segregate 2:2 in at least 18/18 complete tetrads from each cross (with the exception of one tetrad from one cross) and almost all Ts^+ segregants were benomyl resistant (the exceptions presumably having undergone gene conversion or reciprocal recombination in the *ACT1-TUB2* interval).

These *act1-1/ACT1*$^+$ *sac/sac* diploids grew well at 26° but poorly at 36° (Figure 1). As the corresponding *ACT1*$^+$*/ACT1*$^+$ *sac/sac* diploids were very sick even at 26° (Figure 1), *act1-1* is a dominant suppressor at this temperature.

Finally, although the Ts Cs *sac* mutations are dominant for suppression, they are recessive for their Ts

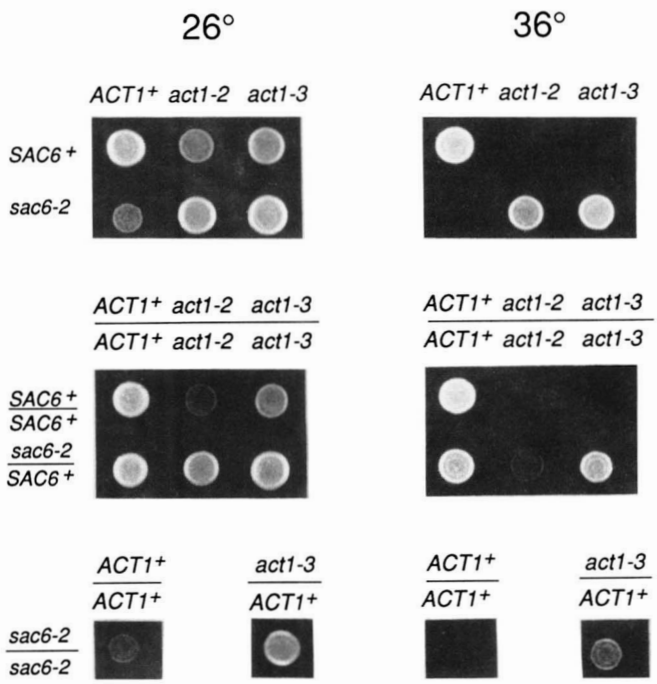

FIGURE 1.—Growth after 39 hr at 26° or 26 hr at 36° of strains carrying various combinations of alleles at the *ACT1* and *SAC6* loci. For each strain, approximately equal numbers of cells were spotted onto YEPD plates (see MATERIALS AND METHODS).

Cs defects, as *sac/SAC*$^+$ heterozygous diploids (at least those carrying the *sac* mutation from mutant 2 or 7) grew well at all temperatures (Figure 1).

Dominant suppressor mutations identify a new gene *SAC6*: In order to determine the number of genes defined by the dominant extragenic suppressor mutations, we tested for linkage among them. It was not possible to assign the mutants to complementation groups, as for most of them (13 out of 17 tested) suppression is the only phenotype, and in all cases suppression is dominant. Ts$^+$ *act1-1 sac* strains were crossed to each other (Table 3), and the segregation of temperature sensitivity was followed in tetrad analysis. Twenty-four suppressors were analyzed in this way, and all were found, either directly or indirectly (Table 3), to be linked to each other. All further analyses were carried out with alleles that were well documented as being in the same linkage group, *i.e.*, from mutants 2, 6, 7, 15 or 19 (Table 3). In order to determine whether this linkage group was the same as *SAC1, 2, 3, 4* or *5* (NOVICK, OSMOND and BOTSTEIN 1989) crosses were made between representative *act1-1 sac* strains. Tetrad analysis (not shown) indicated that the dominant suppressor mutations were not in any of the previously defined *SAC* genes, and thus identify a new gene *SAC6*.

Since the *SAC6* gene yields some mutants with recessive phenotypes, we call the wild-type *SAC6* and the mutants *sac6-n* [where "*n*" indicates allele number and is the same as the mutant number (Table 3)] regardless of the mutant phenotype. Preliminary evidence (our unpublished data) suggests that the null phenotype may be recessive temperature-sensitive growth.

TABLE 3

Linkage analysis of pseudorevertants[a]

Mutant	Crossed to mutant	Total tetrads[b]
1	6, 7, 15, 17, 18, 19, 20, 21, 22, 23, 24	58
2	6, 7, 15, 17, 19	39
3	7, 17, 18, 20	19
4	7[c], 17, 18, 20	19
5	7, 17[c], 18, 20	17
6	1, 2, 7, 15, 19	34
7	1, 2, 3, 4[c], 5, 6, 15, 19	50
8	18, 20	10
9	17	4
10	18	6
11	18	5
12	17	2
13	18	7
14	17[c]	19
15	1, 2, 6, 7, 19	33
16	17	6
17	1, 2, 3, 4, 5[c], 9, 12, 14[c], 16	60
18	1, 3, 4, 5, 8, 10, 11, 13	37
19	1, 2, 6, 7, 15	36
20	1, 3, 4, 5, 8	17
21	1	2
22	1	7
23	1	7
24	1	6

[a] Crosses were made between pairs of *act1-1 sac* strains derived from the diploid revertants listed. In each case, except for the crosses indicated by "*c*" (see below), all segregants were Ts$^+$. Five other mutants (25, 26, 28, 29 and 30) carrying extragenic suppressor mutations (see text), were not analyzed in this way.

[b] The total number of complete tetrads that were analyzed per mutant is shown.

[c] Approximately 1 in 24 segregants were Ts. These indicate the suppressor mutations are either in two tightly linked genes, or are different mutations in the same gene.

***sac6* suppression is allele specific:** Information concerning the mechanism of suppression can sometimes be obtained from studies of allele specificity (BOTSTEIN and MAURER 1982). In particular, the observation that a mutation can suppress some, but not all, mutant alleles, indicates that the suppressor does not simply bypass the requirement for the mutant gene product. Therefore, crosses were made to determine whether the *sac6* mutations could suppress the *act1-2* or *act1-4* mutant alleles. When *tub2-201 act1-1 sac6* strains were crossed to *TUB2*$^+$ *act1-2 SAC6*$^+$ strains (Table 4), temperature sensitivity segregated 2:2, and about 50% of the Ts$^+$ (*sac6*) segregants were found to be benomyl sensitive (*act1-2*). Thus *act1-2*, like *act1-1*, is suppressed by *sac6* mutant alleles (Figure 1). However, when crosses were made between *tub2-201 act1-1 sac6* and *TUB2*$^+$ *act1-4 SAC6*$^+$ strains (Table 4), an excess of Ts segregants was seen. Moreover, all Ts$^+$ (*sac6*) segregants were benomyl resistant (*act1-1*) and those benomyl sensitive (*act1-4*) segre-

TABLE 4

Allele specificity of *sac6* suppression[a]

sac6 allele	Cross to *act1-2*[b] PD	TT	NPD	Ts⁺ Ben^r:Ben^s	Cross to *act1-4*[c] PD	TT	NPD	Ts⁺ Ben^r:Ben^s
sac6-2	0	5	2	5:8	0	7	0	7:0
sac6-6	0	8	0	8:8	1	3	3	5:0
sac6-7	1	5	2	7:9	0	5	2	6:0
sac6-15	2	3	1	7:5	1	5	1	7:0
sac6-19	0	5	0	5:5	0	5	3	4:1[d]

[a] Crosses were made between strain DBY1991 (*TUB2⁺ act1-2 SAC6⁺*), DBY5231 or DBY5232 (*TUB2⁺ act1-4 SAC6⁺*), and various strains harboring different *sac6* mutant alleles (*tub2-201 act1-1 sac6*). Diploids were isolated and sporulated, and tetrads were dissected. Segregants were tested for growth on YEPD plates at various temperatures (14–37°), as well as for benomyl resistance. The ascus types with respect to the *SAC6* and *TUB2* (hence *ACT1*) loci, and the ratio of Ben^r:Ben^s segregants that were Ts⁺, were determined. These data are shown in the respective columns.

[b] Temperature sensitivity segregated 2:2 in each of these crosses. This, together with the fact that about half the Ts⁺ segregants were Ben^s (see adjacent column) indicates that *sac6* suppresses the *act1-2* defect. Therefore PD, TT and NPD are all 2:2 in these crosses.

[c] Temperature sensitivity did not segregate 2:2 in these crosses. Instead, an excess of Ts segregants was seen. This, together with the fact that essentially all the Ts⁺ segregants were Ben^r (see adjacent column) indicates that *sac6* does not suppress the *act1-4* defect. Therefore, PD = $2^+:2^-$; TT = $1^+:3^-$; and NPD = $0^+:4^-$ in these crosses. Except in the cross with the *sac6-15* strain, the *act1-4 TUB2⁺ sac6* segregants (in each NPD or TT ascus) were extremely sick or dead.

[d] The Ts⁺ (*act1 sac6-19*) segregant that was Ben^s presumably carried the *act1-1* mutation as a result of gene-conversion in the *ACT1-TUB2* interval.

gants that were presumed to carry the suppressor mutation yielded extremely small spore colonies or were inviable (Table 4). Thus, *sac6* mutations not only fail to suppress the *act1-4* mutation, but exacerbate its Ts defect. This synthetic phenotype, together with the fact that suppression is allele specific and reciprocal, suggests the *ACT1* and *SAC6* gene products function in related cellular processes, and that *sac6* mutations do not suppress by simply bypassing the function of actin at high temperature.

Crosses were made to determine whether the *sac6* mutations were dominant suppressors of the *act1-2* defect. Thus, *act1-2/act1-2 sac6/SAC6⁺* diploids (carrying *sac6-2, sac6-6* or *sac6-7* mutant alleles) were generated, and tested for growth at 26° or 36°. They were found to grow well at 26°, but poorly or not at all at 36° (Figure 1). As *act1-2/act1-2 SAC6⁺/SAC6⁺* strains grew poorly even at the nominally permissive temperature of 26° (NOVICK and BOTSTEIN 1985; see Figure 1), the *sac6* mutations tested are dominant suppressors at this temperature.

***sac6* suppression is *MOX1* independent:** Three of the five *SAC* genes described previously (NOVICK, OSMOND and BOTSTEIN 1989) require a second gene (*MOX1*) for suppression. Thus, *act1-1 sac MOX1* strains are Ts⁺, whereas *act1-1 SAC⁺ MOX1* and *act1-1 sac mox1-1* strains are Ts. In order to determine whether *sac6* suppression is *MOX1*-dependent, crosses were made between *act1-1 sac6 MOX1* and *act1-1 SAC6⁺ mox1-1* strains, and temperature-sensitivity was followed in tetrad analysis. In each of five crosses (with *sac6-2, sac6-6, sac6-7, sac6-15* or *sac6-19* mutant alleles), suppression segregated 2:2 (not shown). Therefore, the *sac6* mutations tested can suppress *act1-1* in either a *MOX1* or *mox1-1* background. We have not ruled out the relatively unlikely alternative possibility that *SAC6* and *MOX1* are tightly linked genes.

Suppressor analysis of temperature-sensitive *sac6* mutants: The temperature sensitivity of *ACT1⁺ sac6* strains enabled us to look for pseudorevertants of these mutants. Thus, spontaneous, independent Ts⁺ revertants of strain DBY5220 (*sac6-2*), DBY5221 (*sac6-6*) or *DBY5222* (*sac6-7*) were sought and 27 revertants were isolated (see MATERIALS AND METHODS). These were tested for growth at various temperatures (ranging from 11–37°), to determine whether the new mutations suppressed the poor-growth phenotype of the *sac6* strains at all temperatures. All except one of the revertants (DBY5245) were found to grow well at all temperatures (Table 5).

The revertants were then crossed to wild type (strain DBY2059 or DBY2060) to determine whether the suppressors were linked to the *sac6* mutations. Eleven revertants showed poor spore viability or weak suppression in these crosses and were not analyzed further. The remaining 16 revertants are listed in Table 5. Of these, 11 showed tight linkage of the suppressor to the *sac6* mutation (Table 5), and are most likely intragenic revertants. One of these, DBY5245, is potentially useful, as it grows well at 30–37°, but fails to grow at 11–20°. Thus, this mutant (which probably carries two mutations in the *SAC6* gene; see Table 5) has *bona fide* permissive and restrictive temperatures, and so is easier to work with than the parent strain (which presumably carries just a single mutation in *SAC6*). Of the 5 revertants that carried suppressor ("*sup*") mutations unlinked to *SAC6* (Table 5), three yielded Ts Cs⁺ segregants that were distinct from the Ts Cs *sac6* segregants. The patterns of segregation suggested that, in each case, the Ts Cs⁺ strains had the genotype *SAC6⁺ sup* and that the *sup* mutations were unlinked to *SAC6* (not shown). Thus, like the Ts *act1-1* and *sac6* mutations discussed above, these Ts *sup* and *sac6* mutations show reciprocal suppression.

Crosses were made to determine whether the extragenic suppressor mutations were linked to *ACT1*. Thus, *sac6 sup* strains were crossed to *sac6 act1-1* strains, and diploids were isolated and sporulated. In each case, the diploids formed were homoallelic at the *SAC6* locus. Tetrad analysis indicated that 4 of the 5 revertants, including the three with the Ts *sup* muta-

TABLE 5

Ts$^+$ revertants of temperature-sensitive *sac6* mutants

sac6 mutant	Ts$^+$ revertants[a]	*sac6 sup* phenotype	Suppressor linked to			No. tetrads[b]	*SAC6$^+$ sup* phenotype[c]
			SAC6	*ACT1*	Other		
DBY5221 (*sac6-6*)	5236	Ts$^+$ Cs$^+$	−	+	−	11	Ts Cs$^+$
	5238	Ts$^+$ Cs$^+$	+	−	−	8	nr
	5239	Ts$^+$ Cs$^+$	+	−	−	5	nr
	5240	Ts$^+$ Cs$^+$	+	−	−	9	nr
	5241	Ts$^+$ Cs$^+$	−	+	−	10	Ts$^+$ Cs$^+$
	5242	Ts$^+$ Cs$^+$	+	−	−	9	nr
	5243	Ts$^+$ Cs$^+$	+	−	−	10	nr
	5244	Ts$^+$ Cs$^+$	−	+	−	11	Ts Cs$^+$
DBY5220 (*sac6-2*)	5245	Ts$^+$ Cs$^-$	+	−	−	12	nr
	5249	Ts$^+$ Cs$^+$	−	−	+	d	Ts$^+$ Cs$^+$
	5250	Ts$^+$ Cs$^+$	+	−	−	11	nr
	5251	Ts$^+$ Cs$^+$	+	−	−	11	nr
DBY5222 (*sac6-7*)	5253	Ts$^+$ Cs$^+$	+	−	−	11	nr
	5254	Ts$^+$ Cs$^+$	−	+	−	12	Ts Cs$^+$
	5260	Ts$^+$ Cs$^+$	+	−	−	6	nr
	5262	Ts$^+$ Cs$^+$	+	−	−	5	nr

[a] DBY designations are shown.

[b] For those suppressors linked to *ACT1* or *SAC6*, the number of complete tetrads analyzed with respect to the linked gene (indicated by "+") is shown. In all these cases, except with DBY5241 and DBY5245 (see below), no recombinants were seen either in these tetrads or in other, incomplete tetrads. In the cross between DBY5245 and DBY2059, two of the 12 tetrads had a Ts Cs segregant; in the cross between DBY5241 and an *act1-1 sac6-2* strain, one Ts Cs segregant was obtained. It is likely that these all arose by gene conversion, but it is also possible that they are due to recombination between tightly linked genes, and thus that the suppressor mutations in DBY5245 and DBY5241 are in genes *linked* to *SAC6* and *ACT1*, respectively.

[c] For those revertants that are intragenic, this column is not relevant ("nr").

[d] When DBY5249 (*sac6-2 sup*) was crossed to wild-type, *sup* was found to be unlinked to *sac6* (2 PD: 7 TT: 2 NPD). When DBY5249 was crossed to an *act1-1 sac6-2* strain, *sup* was found to be unlinked to *ACT1* (2 PD: 6 TT: 1 NPD).

tions, carried suppressor mutations linked to *ACT1* (Table 5); in each case, all segregants (except one from DBY5241) from at least 10 complete tetrads were Ts$^+$. Thus, the suppressor mutations in these revertants are most likely in *ACT1* and they are designated *act1-7* (from DBY5236), *act1-8* (from DBY5244), *act1-9* (from DBY5254) and *act1-10* (from DBY5241). The three Ts mutations are recessive, and complementation testing with *act1-1* mutant strains confirmed the linkage assignments.

Cloning the *SAC6* gene: Initially, an attempt was made to isolate the *SAC6* gene from a wild-type library by complementation of the Ts defect of an *ACT1$^+$ sac6* mutant strain. However, such strains grow very poorly even at the nominally permissive temperature (see above), so revertants are strongly selected, and it turned out to be impractical to sort through them for Ts$^+$ transformants. For this reason, we decided to generate a library from an *act1-1 sac6-6* strain, and to isolate the mutant *sac6* gene by its dominant suppression of the Ts phenotype of an *act1-1* strain (see MATERIALS AND METHODS). Strain DBY2001 (*act1-1 SAC6$^+$ ura3-52*) was transformed with a genomic library generated in YCp50 [a centromere-containing vector with the selectable marker *URA3* (see MATERIALS AND METHODS)] from strain DBY5218 (*act1-1 sac6-6*). Selection of Ts$^+$ Ura$^+$ transformants yielded one candidate, which was purified by streaking for single colonies on minimal plates lacking uracil at 26°. A culture was then grown under non-selective conditions (YEPD liquid medium at 26°) and was plated for single colonies under the same conditions. Of 144 colonies tested, 7 were Ts Ura$^-$ and 137 were Ts$^+$ Ura$^+$. The Ts$^+$ phenotype of this transformant is therefore unstable and likely to be plasmid-borne, as there is an absolute correlation between the loss of plasmid (the *URA3* marker) and the acquisition of temperature sensitivity.

As the *sac6-6* mutation in the genome suppresses the Ts defect of *act1-1*, but not *act1-4*, mutant strains and is a dominant suppressor (see above) we expected that plasmid carrying the mutant *sac6* gene would also suppress in an allele-specific way. Plasmid was therefore isolated from the original transformant, amplified in HB101, and used to transform strains DBY2001 (*act1-1*) and DBY2326 (*act1-4*). Transformants were selected on minimal plates lacking uracil at 26°, and were tested for growth on YEPD solid medium at 26° or 37°. As expected, 10/12 Ura$^+$ transformants obtained from DBY2001 were Ts$^+$, whereas 10/10 Ura$^+$ transformants obtained from DBY2326 were Ts. We conclude that the plasmid (pRB1275) carries the mutant *sac6* gene. This conclusion was further confirmed by linkage analysis (see below).

Genetic mapping of the *SAC6* gene: *SAC6* was assigned to chromosome *IV* by blot hybridization of OFAGE gels of intact yeast chromosomes (CARLE and OLSON 1985), using a 4.2-kb *Bam*HI-*Sal*I fragment of pRB1275 as probe. To localize the gene more precisely, it was first marked with an integrating plasmid (pRB1276) containing part of the *SAC6* gene on YIp5 (MATERIALS AND METHODS) (ADAMS, BOTSTEIN and DRUBIN 1989). Thus, pRB1276 was cut in the insert (in the unique *Bst*EII site) to direct integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) to *SAC6*, and was then introduced by transformation into strain DBY1827. Ura$^+$ transformants were isolated, and the integration of pRB1276 at the *SAC6* locus of one transformant was confirmed by genomic gel-transfer hybridization analysis (not shown). This transformant strain was then crossed to Ura$^-$ strains carrying multiple chromosome IV markers. Tetrad analysis revealed tight linkage of the integrated *URA3* marker to *aro1* in strain DBY5224 (27 PD: 0 T: 0 NPD). In order to confirm that this is the same as the *SAC6* locus defined by suppression, strain DBY5229 (*act1-1 tub2-201 sac6-6 ARO1$^+$ HOM2$^+$*) was crossed to DBY5228 (*act1-1 TUB2$^+$ SAC6$^+$ aro1 hom2*), and the segregation of the various markers was followed in tetrad analysis. Tight linkage was seen between *sac6* and *ARO1$^+$* (44 PD: 2 TT: 0 NPD). As the recombinant segregants in the two tetratypes showed recombination between *aro1* and *hom2*, the order of these three genes on the chromosome is *ARO1 SAC6 HOM2*, with *SAC6* approximately 2 cM from *ARO1*.

DISCUSSION

Suppressor analysis has long been recognized as a useful means to identify interacting gene products [see BOTSTEIN and MAURER (1982) and HUFFAKER, HOYT and BOTSTEIN (1987) for reviews relevant to the yeast cytoskeleton]. The primary result of this paper is the discovery of a new gene (*SAC6*) that not only yields dominant suppressors of actin mutations, but also the observation that some of these alleles show apparently recessive growth defects that are suppressed by the same actin mutations. This phenomenon of "reciprocal suppression" may represent a new and possibly quite general way to discover genes whose products interact.

In a companion study, NOVICK, OSMOND and BOTSTEIN (1989) described pseudorevertants carrying extragenic suppressors of the Ts *act1-1* mutation. These suppressors acquired a new cold-sensitive lethal phenotype along with their ability to suppress the Ts phenotype of *act1-1*. It was the similarity between the *sac* and *act1* mutant phenotypes, along with the allele specificity of suppression, that made the idea of interaction at the level of gene product most attractive. In the present case, we have not yet studied the phenotypes of the new suppressors, but we have observed an unusual genetic phenomenon: allele-specific reciprocal suppression. The allele-specificity is true in both directions: only the *act1-1* and *act1-2* alleles are suppressed by a given *sac6* allele, and equally significantly, *act1-4* does not suppress the *sac6* alleles that have growth defects.

The observation that *sac6* and *act1* mutations can suppress each other's defects provides unusually strong genetic evidence for the idea that the product of the *SAC6* gene interacts directly with actin. The reciprocity alone makes particularly unlikely trivial mechanisms that could easily account for unidirectional suppression. A suppressor mutation that altered the intracellular milieu, for example, would not normally cause a growth defect only when separated from the original mutation. Further evidence that the *SAC6* gene product interacts directly with actin comes, of course, from the observation that suppression is allele specific in both directions.

Several combinations of *act1* and *sac6* mutant alleles not only fail to suppress, but exacerbate each other's Ts phenotypes. This result is reminiscent of the synthetic lethality observed by NOVICK, OSMOND and BOTSTEIN (1989) for certain combinations of recessive *sac* suppressor alleles and *act1* mutations. Although it is possible in this case that the Ts defects are simply additive, it seems more likely, in light of the fact that suppression is reciprocal and allele specific, that more specific incompatibilities are involved.

Different isolation schemes yielded different suppressors: The isolation of *sac6* suppressors differed from the isolation of the *sac1-sac5* suppressors (NOVICK, OSMOND and BOTSTEIN 1989) in two important ways. First, we looked here specifically for *dominant* suppressors (previous *sac* mutations are all recessive) and second, we extended our analysis to suppressors that are not temperature conditional for growth in the presence of the *act1-1* mutation (previous *sac* mutants all had, by design, the Cs growth phenotype in addition to the suppression phenotype). These two differences in the isolation scheme resulted in the recovery of a completely nonoverlapping set of mutants. Thus, 24 of 24 extragenic suppressors analyzed were found to be linked to each other, and this linkage group was found to be different from the five *SAC* genes identified previously. These results suggest that the search for explicitly dominant suppressors should be considered whenever pseudoreversion analysis is undertaken.

Why were no alleles of *SAC1-SAC5* obtained in this study and why was *SAC6* not identified previously? To address the first question, it is clearly relevant that recessive suppressors were excluded from the analysis; however, it is interesting that no dominant suppressor mutations in any of the five genes were obtained.

Perhaps the gene products function in a way that precludes the isolation of dominant suppressor mutations; for example, in order for such a gene product to function, it might be necessary for it to interact with actin along the entire length of the polymer. If this were the case, and if mutant *sac* gene product only were able to interact productively with mutant actin, then the presence of wild-type *SAC* gene product in a heterozygous diploid cell might be inhibitory; the suppressor mutation would then be recessive.

At first glance, it seems equally curious that *SAC6* was not identified previously, as dominant suppressors were not excluded from that analysis (NOVICK, OSMOND and BOTSTEIN 1989). It may be relevant that none of the revertants analyzed in the present study are cold sensitive in the presence of the *act1-1* mutation; it may therefore just be improbable to obtain such mutants in this gene by the method of NOVICK, OSMOND and BOTSTEIN (1989). It is these idiosyncratic behaviors of particular genes that may be the strongest argument for using as great a variety of genetic tricks as possible to isolate mutations in interacting genes, including, now, searches for reciprocal suppressors.

The screening of revertants for those that, in addition to suppression, have a phenotype similar to that of the original mutant, has proven to be a useful tool for identifying those revertants most likely to carry mutations in genes encoding interacting proteins (JARVIK and BOTSTEIN 1975; MOIR *et al.* 1982; BOTSTEIN and MAURER 1982; NOVICK, OSMOND and BOTSTEIN 1989). The primary advantage of this method is the ability to study the new gene via the new phenotype. It is important to note that this advantage is also a feature of reciprocal suppression, for the new gene can be studied by the phenotype revealed when the reciprocal suppressor allele is removed.

Reciprocal suppression yields new actin mutations: The suppressor analysis of Ts *sac6* mutants was interesting in a number of ways. First, the observation that several of the revertants carried suppressor mutations in *ACT1* provided additional evidence that *act1* and *sac6* mutations can suppress each other's defects. Second, of the four new actin mutations isolated in this way, three are temperature-sensitive in a *SAC6*$^+$ background. These are almost certainly different from *act1-1, act1-2* or *act1-4*, as the new alleles grow better at the nominally permissive temperature (26°), and are more leaky at the restrictive temperature (36°). They therefore add diversity to the collection of temperature-conditional actin mutations already available, and demonstrate an alternative way to identify new alleles. This is particularly relevant in light of the difficulty of obtaining temperature-conditional actin mutations in yeast (SHORTLE, NOVICK and BOTSTEIN 1984; T. DUNN and D. SHORTLE, personal communication). Moreover, as we turn our attention to more biochemical analyses of the yeast actin cytoskeleton, non-temperature-conditional mutant actins identified on the basis of suppression [such as that in DBY5241 (Table 5)] will undoubtably also prove to be of value.

Finally, in addition to the four *act1* mutations identified by selecting pseudorevertants of Ts *sac6* strains, a mutation in still another gene was obtained by the isolation of revertant DBY5249 (Table 5). Although there is no evidence yet that this suppressor mutation is in a gene whose product interacts directly with the *SAC6* gene product, it indicates that it may be possible to identify such genes.

This work is dedicated to S. V. ADAMS. We are especially indebted to JOHN PRINGLE for his insistence that screening for cold-sensitivity might not be absolutely necessary. We thank G. FINK, D. SHORTLE and T. DUNN for strains. We are particularly indebted to all our colleagues in the laboratory for helpful discussions, and especially thank K. HENNESSEY, T. STEARNS, D. DRUBIN and C. CHAN for advice and comments on the manuscript. Research was supported by grants to D.B. from the National Institutes of Health (GM21253 and GM18973) and the American Cancer Society (MV90). A.A. is a Burroughs Wellcome Fund Fellow of the Life Sciences Research Foundation, and gratefully acknowledges their support.

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Communicating editor: M. CARLSON