Mutations in the SAC1 Gene Suppress Defects in Yeast Golgi and Yeast Actin Function

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Abstract. The budding mode of Saccharomyces cerevisiae cell growth demands that a high degree of secretory polarity be established and directed toward the emerging bud. We report here our demonstration that mutations in SAC1, a gene identified by virtue of its allele-specific genetic interactions with yeast actin defects, were also capable of suppressing sec14 lethals associated with yeast Golgi defects. Moreover, these sac1 suppressor properties also extended to sec6 and sec9 secretory vesicle defects. The genetic data are consistent with the notion that SAC1p modulates both secretory pathway and actin cytoskeleton function. On this basis, we suggest that SAC1p may represent one aspect of the mechanism whereby secretory and cytoskeletal activities are coordinated, so that proper spatial regulation of secretion might be achieved.

The secretory pathway represents a major route of intracellular protein traffic in eukaryotic cells. It is distinguished by a set of membrane-enclosed organelles that harbor activities associated with the transport and sorting of proteins from the cytoplasm to various other intracellular destinations and the cell surface (Palade, 1975). Consequently, the secretory pathway plays an essential role in maintaining the proper level of biochemical compartmentation within the cell. The tractability of Saccharomyces cerevisiae to genetic and biochemical analysis has made yeast a powerful experimental model with which to study the molecular basis of secretory pathway function (reviewed in Schekman, 1982). To date, some 27 genes have been identified whose gene products are necessary for exocytic flow from the cytoplasm to the cell surface (Novick et al., 1980; Deshaies and Schekman, 1987; Newman and Ferro-Novick, 1987). These genes have been defined by conditional-lethal mutations that cause defects at specific stages of the secretory pathway.

In addition to such essential functions, several other genes have been defined whose products influence yeast exocytic processes. For instance, the yeast cytoskeleton appears to impose a level of spatial organization to the secretory pathway. During the budded portion of the cell cycle, yeast cell surface growth and secretion are directed primarily to the bud (Tkacz and Lampen, 1973; Field and Schekman, 1980). This polarity coincides with the asymmetric organization of cellular actin (Kilmartin and Adams, 1984; Adams and Pringle, 1984), thereby implicating the actin cytoskeleton as the mediator of such a spatially restricted mode of yeast cell growth. Corroborative evidence for the role of actin cytoskeleton in yeast cell surface organization has been obtained from phenotypic analyses of conditional-lethal actin mutations (Novick and Botstein, 1985). The mechanisms where-
Table 1. Yeast Strains

| Strain       | Genotype                        | Origin                      |
|--------------|---------------------------------|-----------------------------|
| CTY182*      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub> | Bankaitis et al. (1989)     |
| CTY1-1A      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, sec14-1<sup>±</sup> | Bankaitis et al. (1989)     |
| CTY2-1C<sup>±</sup> | MATα, ade2-101<sub>obs</sub>, sec14-1<sup>±</sup> | This study                 |
| CTY100*      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, sec14-1<sup>±</sup>, sac1-26<sup>±</sup> | This study                 |
| CTY101*      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, sec14-1<sup>±</sup>, sac1-27<sup>±</sup> | This study                 |
| CTY108*      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, sec14-1<sup>±</sup>, sac1-28<sup>±</sup> | This study                 |
| CTY109*      | MATα, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, sec14-1<sup>±</sup>, sac1-29<sup>±</sup> | This study                 |
| CTY151      | MATα, ade2-101<sub>obs</sub>, ura3-52, Δhis3-200, sec14-1<sup>±</sup>, sac1-18<sup>±</sup> | This study                 |
| YPS4        | MATα, ade2-101<sub>obs</sub>, ura3-52, Δhis3-200, lys2-801<sub>um</sub>, ΔTrp1 | Phil Hieter               |
| CTY73        | MATα, ade2-101<sub>obs</sub>, ura3-52, Δhis3-200, ΔTrp1, sec14-1<sup>±</sup> | This study                 |
| CTYD39       | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-1<sup>±</sup>, sac1-26<sup>α</sup> | This study                 |
| CTYD40       | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-1<sup>±</sup>, sac1-27<sup>α</sup> | This study                 |
| CTYD41       | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-1<sup>±</sup>, sac1-28<sup>α</sup> | This study                 |
| CTYD42       | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-1<sup>±</sup>, sac1-29<sup>α</sup> | This study                 |
| CTYD61<sup>±</sup> | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-129::HIS3, sac1-26<sup>α</sup> | This study                 |
| CTYD62<sup>±</sup> | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-129::HIS3, sac1-27<sup>α</sup> | This study                 |
| CTYD63<sup>±</sup> | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-129::HIS3, sac1-28<sup>α</sup> | This study                 |
| CTYD64<sup>±</sup> | MATα<sub>α</sub> _ADE2_ ura3-52, Δhis3-200, lys2-801<sub>am</sub>, ΔTrp1, sec14-129::HIS3, sac1-29<sup>α</sup> | This study                 |

* Designated yeast strain is isogenic to CTY1-1A.
† CTY2-1C was constructed in the same cross that generated CTY1-1A (Bankaitis et al., 1989), and was obtained from the same tetrad that yielded CTY1-1A.
§ Diploid strains CTYD61, CTYD62, CTYD63, and CTYD64 are isogenic to strains CTYD39, CTYD40, CTYD41, and CTYD42, respectively. We also note that sec14-129::HIS3 is a redesignation of the sec14::HIS3 allele that was previously described (Bankaitis et al., 1989).

Bethesda Research Laboratories (Gaithersburg, MD) and Pharmacia Fine Chemicals (Piscataway, NJ). Electrophoresis reagents were from Bio-Rad Laboratories (Richardson, CA), and all materials for invertase assays were obtained through Sigma Chemical Co. (St. Louis, MO). α-[32P]dATP (800 Ci/mmole) and α-[32P]UTP (3,000 Ci/mmole) were bought from Amersham Corp. (Arlington Heights, IL).

### Transformations

Yeast were transformed to Ura<sup>+</sup> or His<sup>+</sup>, with plasmid or linear DNA, on minimal SD media by the lithium acetate method (Ito et al., 1983). DNA for ampicillin resistance with plasmid DNA by the calcium chloride procedure (Maniatis et al., 1982).

### DNA Sequencing

Nucleotide sequence analysis used the chain termination method of Sanger et al. (1977). Template DNAs were generated by subcloning appropriate restriction fragments into pTZ phagemid vectors (Mead et al., 1986). Phage M13K07 was used as helper for template synthesis.

### Isolation of Suppressors

The sec14<sup>±</sup> strains CTY1-1A and CTY2-1C were streaked for isolation on YPD plates at 30°C. 100 isolated colonies from each strain were picked and patched onto YPD plates. After a 72-h incubation at 35°C, one spontaneous Ts<sup>+</sup> variant was picked from each patch that exhibited such revertants. Of the 100 CTY2-1C patches, 58 yielded at least one Ts<sup>+</sup> revertant, whereas 49 of the 100 CTY1-1A patches were productive. These revertants were purified at 25°C and rechecked for the Ts<sup>+</sup> phenotype. In this manner, 107 independent revertants of sec14<sup>±</sup> were obtained.

### Phenotypic Evaluations of Suppression and Cold Sensitivity

The phenotypic scoring of suppression (Ts<sup>+</sup>), nonsuppression (ts), and cold sensitivity (cs, at 13°C) was performed on both YPD and minimal SD media. The ability of test strains to form single colonies by 48 h at 37°C distinguished the Ts<sup>+</sup> from the ts phenotypes. Similar criteria were used for scoring of Cs<sup>+</sup> versus cs at 13°C except that 12 d were allowed for growth at that low temperature.

### Invertase Secretion

The procedures for measuring total and extracellular invertase in yeast have been described (Bankaitis et al., 1989). Minor modifications are noted in the text. Invertase was assayed by the published method of Goldstein and Lampen (1979). Invertase units were expressed as nanomoles glucose produced per min at 30°C.

### Northern and Southern Analyses

Yeast genomic DNA was obtained from 5 ml cultures as described (Sherman et al., 1983). The digested DNA was transferred to Gene Screen Plus (DuPont, NEN Research Products, Wilmington, DE) according to the manufacturer's specification. Filters were prehybridized at 55°C in 50% formamide, 5× SSPE (50 mM NaI-I2PO₄, 750 mM NaCl, 5 mM EDTA), 1% SDS, 250 μg/ml nonhomologous DNA, and 500 μg/ml tRNA for 12 h. Radioactive antisense probes, generated by in vitro transcription of the noncoding SEC14 or RIS13 strands with phage T7 RNA polymerase and [75P]UTP (Nielsen and Shapiro, 1986), were added and hybridization was allowed to occur for 24 h at 55°C with shaking. The filters were washed...
twice at 25°C with 2× SSPE, 1% SDS followed by two 30-min washes at 65°C in 0.2× SSPE, 1% SDS. The filters were evaluated by autoradiography.

Yeast RNA was prepared from 5-ml cultures by the glass bead method of Elder et al. (1983). Total RNA was quantitated spectrophotometrically, fractionated on formaldehyde-1% agarose gels, and transferred to Gene Screen Plus filters. These filters were treated as described above for the Southern analyses except that the prehybridization and hybridization reactions were incubated at 62°C, and the final two posthybridization washes were at 70°C in 0.1× SSPE, 1% SDS.

**Calcifluor Staining**

Yeast cells were grown to early logarithmic phase and shifted to the desired temperature for the appropriate period of time (see text). The cells were subsequently fixed with formaldehyde (final concentration 3.7%) for 2 h at the postshift temperature, washed three times in 35 mM potassium phosphate buffer (pH 6.8), 0.5 M MgCl₂, and finally resuspended in 50 mM potassium phosphate (pH 7.5). Calcifluor White M2R (Sigma Chemical Co.) was added to a final concentration of 1 µg/ml, and allowed to react for 5 min at room temperature. The cells were subsequently washed and mounted on glass slides essentially as described by Adams and Pringle (1984). Chitin staining was recorded on TMAX400 film (Eastman Kodak Co., Rochester, NY) via photography using an Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY) equipped for fluorescence with UV filters and an HBO 50-W mercury lamp.

**Results**

**Identification of an Extragenic Suppressor of sec14-1<sup>ts</sup> that has Simultaneously Acquired a Cold Sensitive Phenotype for Growth**

The four previously described sec14<sup>ts</sup> mutations are all the result of a G to A transition that converts codon 266 of the 304-codon SEC14 from GGT (Gly) to GAT (Asp). We refer to this lesion as sec14-1<sup>ts</sup>. The sec14-1<sup>ts</sup> nonsense mutation profoundly decreases SEC14p function under restrictive conditions, as judged by the recessive ts-lethal phenotype. This function is not totally destroyed, however, because sec14-1<sup>ts</sup> is dosage suppressible (manuscript in preparation). Because inspection of the SEC14 nucleotide sequence and the inferred SEC14p primary sequence failed to suggest some known biochemical activity that may be associated with SEC14p function (Bankaitis et al., 1989), we undertook a pseudoreversion analysis of sec14-1<sup>ts</sup> to identify gene products that either modulate SEC14p activity or are responsive to it. The conditional-lethal phenotype of sec14-1<sup>ts</sup> provided a selection for Ts<sup>+</sup> revertants, and we independently isolated a total of 107 such spontaneous revertants from two sec14<sup>ts</sup> haploid strains, CTY11Aa and CTY2-1Cc (see Materials and Methods and Table I).

Dominance tests were performed by crossing suppressor mutants derived from CTY2-1Cc to parental CTY1-1Aa and vice versa. The growth properties of the resultant 107 diploids were then scored at 36°C. In 26 cases, diploids exhibited the parental ts phenotype. This behavior identified the corresponding suppressors to be recessive mutations. The remaining 81 diploids were phenotypically Ts<sup>+</sup>, identifying those corresponding suppressors as dominant mutations. Standard complementation analyses demonstrated that all 26 recessive suppressor mutations fell cleanly into four complementation groups that we have designated rsd1 through rsd4 (recessive suppressor of a secretory defect). These complementation groups were represented by 14, 7, 3, and 2 mutants, respectively. The rsd2, rsd3, rsd4, and dominant suppressor strains had no additional phenotypes other than suppression of sec14-1<sup>ts</sup>. Their characterization will be described elsewhere.

Our initial efforts were directed at characterizing the rsd1 mutants because a screen for unselected phenotypes revealed that all 14 members of rsd1 had simultaneously acquired a new cs phenotype, in addition to the selected Ts<sup>+</sup> suppressor phenotype. Standard meiotic segregation analyses confirmed that the same mutation was responsible for both phenotypes. Data from 20 tetrads, obtained by sporulation of sec14<sup>ts</sup> homozygous diploids that were heterozygous for rsd1<sup>α</sup>/RSDI, demonstrated an absolute cosegregation of the cs and Ts<sup>+</sup> phenotypes. Further genetic analyses clearly showed an independent assortment of sec14-1<sup>ts</sup> and rsd1<sup>α</sup> during meiosis.

**Nucleotide Sequence of RSD1**

A convenient feature of the rsd1<sup>α</sup> mutations was that both the cs and suppressor phenotypes were recessive. This permitted recovery of RSD1 clones by complementation of cs, and allowed routine analysis of complementation by following a second, more rapidly scorable determinant, loss of sec14-1<sup>ts</sup> suppression. Strain CTY151 (MATα, ura3-52, sec14<sup>ts</sup>+, rsd1-3<sup>α</sup>) was transformed with a yeast genomic DNA library propagated in YCp50. The desired transformants were identified by simultaneous selection for Ura<sup>+</sup>, Cs<sup>+</sup>. From an estimated 1 × 10<sup>4</sup> potential Ura<sup>+</sup> transformants, two Cs<sup>+</sup> transformants were isolated. As expected for true products of complementation, both Cs<sup>+</sup> transformants had acquired an unselected ts phenotype. Linkage of Cs<sup>+</sup> to the plasmid was confirmed by recovering the plasmids from E. coli and demonstrating full complementation of the cs and suppressor phenotypes upon transformation of plasmid back into the ten sec14-1<sup>ts</sup>, rsd1<sup>α</sup> strains that were tested.

A physical map of one such plasmid insert (pCTY100) is shown in Fig. 1. A series of deletion mapping and subcloning analyses localized the putative RSD1 gene to a 2.4-kb Xba I restriction fragment. The Bam HI, Pvu II, Eco RI, Xho I, and the two Bcl I sites that reside within the minimal complementing Xba I fragment were also determined to lie within the presumed RSD1 gene. Various restriction fragments, derived from internal regions of the RSD1 gene, were used to program in vitro synthesis of radiolabeled RNA of known polarity for the probing of blots of total yeast RNA. These Northern analyses identified a single 2.2-kb species that was transcribed in the Xho I to Bam HI direction (see Fig. 1) and exhibited a steady-state abundance that was approximately one-half that of TRP1 mRNA (unpublished data).

A genetic proof that pCTY100 represented an authentic RSD1 clone was forthcoming from two independent lines of evidence: (a) rescue of rsd1<sup>α</sup> phenotypes by recombination of a disruption allele of the cloned gene into RSD1/rsd1<sup>α</sup> heterozygous diploids, and (b) marker rescue of a functional RSD1 gene from rsd1<sup>α</sup> mutants transformed with noncomplementing fragments of cloned yeast DNA. For the first allelism test, the yeast HIS3 gene was subcloned into the Pvu II site of the complementing gene and shown to destroy its properties of phenotypic complementation. Because the HIS3 cassette contained no Xba I sites, this disruption plus

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flanking sequences was excised by restriction with endonuclease Xba I and recombined, by transformation with linear DNA to His*, into the genome of a homozygous Δhis3-200, sec14-1Δ diploid. This diploid also exhibited a heterozygous rsd1Δ/RSD1 configuration. Some one-third (7/20) of the His* transfectants acquired an unselected cs, Ts* phenotype that was fully reverted to Cs*, ts by reintroduction of pCTY100 into these strains. These data strongly suggested that the His* transfectants that scored cs, Ts* had experienced a disruption of RSD1. The second line of evidence was obtained by using a strategy that fine structure mapped selected rsd1 mutagenesis to a defined region of cloned DNA. The rsd1 strains CTY108 and CTY109 (see Table I) were transformed to Ura* with YEp24 and YE24-derivative plasmids carrying any one of several noncomplementing DNA segments derived from the presumptive 2.4-kb Xba I minimal complementing fragment. Purified transformants were patched, replica plated onto uracil-deficient media, and incubated under restrictive conditions (13°C). After 10 d, the number of Cs* revertants was estimated for each transformant and compared with the YEp24 control. A significant increase in reversion to Cs* over background was considered to indicate marker rescue of RSD1. For both strains CTY108 and CTY109, multicopy presentation of the distal 1-kb Eco RI-Xba I fragment was necessary and sufficient to yield an estimated two-order of magnitude increase in the frequency of reversion to Cs* over background (some 500-600 revertants per patch as compared with 6-10, respectively). Presentation of noncomplementing sequences outside of this Eco RI-Xba I fragment failed to increase the reversion frequency over that of background. The ability to rescue RSD1 by recombination or gene conversion between rsd1Δ and a noncomplementing cloned DNA established genetic linkage between the cloned DNA and the mutant gene. This result, when coupled with the first allelism test, provided strong proof that an authentic RSD1 clone had been identified.

The nucleotide sequence of the minimal complementing fragment was determined and is shown in Fig. 2. The largest potential open reading frame within the 2.4-kb Xba I fragment spanned 623 continuous codons (Mr = 71,132) and exhibited the correct polarity as inferred from the RSD1 direction of transcription experiments (see above). This open reading frame also spanned the restriction sites that had been determined to reside within RSD1 (i.e., Bam HI, Pvu II, Eco RI, Xho I, and the two Bcl I sites). The carboxy-terminal end of the RSDlp was marked by tandem UAA and UGA termination codons. Since the initiator AUG for the putative 623-amino acid RSDlp lay immediately adjacent to the boundary Xba I site (Fig. 2) we felt it unlikely that this represented the initiator codon in vivo. This configuration also raised the possibility that the entire open reading frame representing the RSDlp may not have been identified but, rather, only a functional, carboxy-terminal segment was obtained. We have determined the nucleotide sequence of genomic DNA that lies upstream of the boundary Xba I site, and found that the open reading frame did not extend beyond that Xba I site into these adjoining sequences (not shown). Thus, the inferred RSDlp primary sequence represented that of the full-length gene product. At present, the best candidate for the initiator is the second AUG. The primary translation product initiating at this AUG was predicted to contain 565 amino acids (Mr = 64,993). The generation of RSDlp-specific antisera will help resolve the question of which initiator codon is actually used.

Regardless of whether the first or the second AUG was the true initiator codon, several features of the RSDlp were revealed upon inspection of the inferred primary sequence. First, RSDlp exhibited a rather hydrophilic character (~23% charged amino acids), and a net positive charge at neutral pH. The calculated pl values for the potential 71- and 65-kD RSDlp forms were 7.6 and 8.3, respectively. Second, we were unable to recognize any likely signal peptide structures that might have served to direct RSDlp into the yeast secretory pathway. Finally, we noted that the net basic character of RSDlp was a function of the carboxy-terminal 95 amino acids. This basic region (pI = 9.8) was separated from the rest of RSDlp by a 15-residue stretch, exhibiting a total of 4 prolines, followed by a run of 23 uncharged residues that constituted the best candidate for a potential membrane spanning element in the protein (Fig. 2). Such analyses suggested that RSDlp might exhibit a small, basic, COOH-terminal do-

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**Figure 1.** Physical map of the yeast RSD1 gene. The 11-kb genomic insert of the rsd1Δ complementing plasmid pCTY100 is given. The RSD1 coding region and direction of transcription are indicated by the solid arrow. For reasons given in the text, the coding region is considered to start at the second Met of the open reading frame. Restriction site abbreviations include: Sst I (S), Pvu II (P), Bam HI (B), Bgl II (Bg), Xba I (X), Bcl I (Bc), Xho I (Xh), Eco RI (E), and Hind III (H). The arrangement of the Xho I and Bcl I sites depicted in the enhancement of the minimal complementing 2.4-kb Xba I fragment (see text), apply only to that fragment. We did not determine the distribution of these two sites in the flanking genomic regions.
Figure Cleves et al. AAATTT TCC AAG CC~ ~&? ~T TTAA.qA ~GA
TGATTTCA}T AGGGCTTCAC CCACTAAA~ TCCATGGA~A CCTTCTTT~T TGTATTTTTC &GCATCGTCC CTGGTGGTAA TTCCCGATAG AGCAATTA~A AGNSCAT~T TTGGAATA~A
TTCT&CC~G TTACTGGTGG TATTTAGG~ TACGTTGAAT GAATGCAGGT CCCTATTATT GACACCTA~A ACTTTAGC~C C~JSTTT~?Ac-a

CTA

Aim Lye Phe Set Lye Pro Asp Pro Leu Lye Arg

ATT CAG TTT GTC AAC TGG CCT AAG T}A GTA

Doolittle, 1982) is boxed. We have arbitrarily designated nucleotide +1 to be at the second ATG in the largest open reading frame as we

at -174. The inferred primary sequence between these two ATO codons is given in lower case to indicate our uncertainty as to whether

TTA YCT AAC

I~u Set Amn

80O

Phe Asp Gin Gin

TTT GAC CAG ~ AAG GAG T}A

Leu Gln Thr

CCT GTT

• CC GAA TAT

I4u

Phe Arg Arg

Thr Ile Phe PlwJPro Ly8 Asp Arg Phe Thr

ACA

Asp Ar~ Thr

~ AGA CC* CA} ~CC =C, TCT ATC ~ ~C0

Thr Ar~ Leu GIF Ala Phe ASh Asp Phe

TTG TTA

Leu Leu ThE Ser Tyr Gln Agn Leu

370 380 390 400 410 420 430 440 450 460 470

CCT GTC AAC ATG TAT TAT TAT GCT TAT ATC~ CAT GAA TGT ~GT AAG ATG C~A TGG CAT AG~ GTG AAA TTG ~TA AT7 ~A7 CAC CTG GAG AA~ TTA GGT

2. Suppression of Golgi and Actin Dysfunction

370 380 390 400 410 420 430 440 450 460 470

TCC CTT AGG TAT TCT CAT AGC GTG CCC TCT GTG ATT AAC CAC AGG GTC TTC ATC CAT

AAAC GAG TAT TCT TCT GTT GTT ATT AAC CAC AGG AAC TAT TAT TAT CAC AAT

370 380 390 400 410 420 430 440 450 460 470

CCT GTC AAC ATG TAT TAT TAT GCT TAT ATC~ CAT GAA TGT ~GT AAG ATG C~A TGG CAT AG~ GTG AAA TTG ~TA AT7 ~A7 CAC CTG GAG AA~ TTA GGT

2. Suppression of Golgi and Actin Dysfunction

370 380 390 400 410 420 430 440 450 460 470

TCC CTT AGG TAT TCT CAT AGC GTG CCC TCT GTG ATT AAC CAC AGG GTC TTC ATC CAT

AAAC GAG TAT TCT TCT GTT GTT ATT AAC CAC AGG AAC TAT TAT TAT CAC AAT

Figure 2. Nucleotide sequence of RSD1. The DNA sequence of the RSD1 coding strand of the 2.4-kb minimal complementing Xba I fragment and the predicted RSD1 primary sequence are shown. Hallmark restriction sites that are referred to in the text are also shown. The proline-rich region is underlined, and the best candidate for a membrane-spanning region (Kyte hydropathy value = 2.606; Kyte and Doolittle, 1982) is boxed. We have arbitrarily designated nucleotide +1 to be at the second ATG in the largest open reading frame as we currently believe this to be the most likely candidate for the initiator codon (see text). The first ATG in the open reading frame is indicated at -174. The inferred primary sequence between these two ATG codons is given in lower case to indicate our uncertainty as to whether it is represented in the RSD1 sequence. For the purposes of this report, the inclusion or exclusion of this sequence is irrelevant (see text).
main. Comparisons of the RSD1 nucleotide and inferred primary sequences to those in the GenBank and PIR data bases failed to reveal informative homologies when the FASTP algorithm of Lipman and Pearson (1985) was applied (ktup = 1 and ktup = 2 modes).

**RSD1 and SAC1 Are Allelic**

We noted that the RSD1 restriction map (Fig. 1) was remarkably similar to that deduced for SAC1, a gene originally recognized by virtue of its allele-specific genetic interactions with yeast actin defects (Novick et al., 1989). Because actin plays some role in the late stages of the yeast secretory pathway (Novick and Botstein, 1985), we investigated the possibility that rslα and sac1 were allelic, and that mutations in a single locus could suppress both secretory (sec1α) and actin (act1-1) dysfunction.

The most convincing demonstration of an identity between RSD1 and SAC1 would have involved a complete nucleotide sequence identity between the two genes. Since the nucleotide sequence of SAC1 is not known (Novick et al., 1989), we established the allelic nature of RSD1 and SAC1 by the cumulative weight of five different lines of evidence. First, we found that mating of rslα and sac1 haploids yielded diploids that retained the parental Cs phenotypes. This failure to complement was observed for all ten rslα and two sac1 alleles that were tested. Second, introduction of a single copy of RSD1 into sec1-6 and sac1-8 strains yielded transformants that had uniformly acquired an unselected Cs phenotype, and third, recombination of RSD1 disruption alleles into sac1+/SAC1 heterozygous diploids gave rise to transformants exhibiting unselected Cs phenotypes.

The fourth line of evidence was obtained from integrative mapping experiments that established a tight genetic linkage between rslα and SAC1. Plasmid pNB50, a YIp5 (SAC1) integrating plasmid constructed by Novick et al. (1989), was linearized at a unique Xho I site that resides within the SAC1 gene. This linearized pNB50 was then used to transform strain CTY73 (MATα, ura3-52, sec14-1, RSD1) to Ura⁰, yielding haploid strain CTY97. Southern analysis of genomic digests of CTY97 verified that the expected integration of pNB50 at the SAC1 locus had occurred in this strain (data not shown). Thus, CTY97 represented a strain whose SAC1 locus was marked with the YIp5-borne URA3 gene. CTY97 was subsequently mated to CTY100 (MATα, ura3-52, SEC14-1, rslα). The purified diploid strain was induced to sporulate and the resultant meiotic progeny that were derived from four-sporo asci were analyzed by standard tetrad analysis. As expected for a single-site integration of pNB50, analysis of a total of 20 asci revealed that all 20 exhibited a 2Ura⁰:2Ura⁻ segregation. Moreover, we found all URA3 progeny to score Ts⁻, Cs⁻ (indicating coinheritance of URA3 with RSD1), whereas all ura3 progeny scored Ts⁺, cs⁺ (indicating URA3 did not cosegregate with rslα). This pattern of URA3 segregation with respect to rslα indicated that the URA3 marker of CTY97 was tightly linked to RSD1. Because URA3 marked the SAC1 locus in this cross, it followed that RSD1 and SAC1 were tightly linked. The cumulative weight of these four lines of evidence provided formal genetic proof of the RSD1/SAC1 allelism.

The fifth line of evidence stemmed from the test of whether suppressor alleles generated in response to actin dysfunction could also suppress sec14-1α. To do this, we followed the phenotypic segregation of Ts⁺:ts in a meiotic analysis of a sec14-1/XSC14, SAC1/sec14-1α diploid. Non-suppression of sec14-1α by sec1-6 would uniformly yield tetradts expressing a 2Ts⁻:2Ts⁺ segregation. In contrast, suppression would yield tetradts that also exhibited segregations of 3Ts⁻:1ts and 4Ts⁻:0ts. The data indicated that sec1-6α suppressed sec14-1α efficiently. Suppression was demonstrated by an analysis of ten tetrads derived from the described diploid. Six tetrads exhibited a 3Ts⁻:1ts segregation, three exhibited a 4Ts⁻:0ts segregation, and one showed a 2Ts⁻:2ts pattern (all Cs progeny were Ts⁺). The suppression efficiency was inferred from our observation that all sec14-1α, sec1-6α double mutants exhibited wild-type growth at 37°C.

Taken together, these results demonstrated that RSD1 was allelic to SAC1, and that mutations in this gene could simultaneously suppress both sec14-1α and actin defects. For purposes of convention we have adopted SAC1 nomenclature for RSD1. Thus, sec1-1α through sec1-15α represent alleles generated in response to act1-1α (Novick et al., 1989), whereas sec1-16α through sec1-29α represent alleles generated in response to sec14-1α.

**Phenotypic and Genetic Properties of sec14-1α-suppressing Alleles of SAC1**

Because the sec14-1α and act1-1α-suppressing alleles of SAC1 were obtained by what appeared to be completely independent selections, we investigated whether the low-temperature (13°C) phenotypes of mutants of the former class were similar to those exhibited by mutants of the latter (as reported by Novick et al., 1989). Our data indicated that there was no obvious phenotypic distinction between these two classes. First, only quite minor increases in intracellular levels of secretory invertase were observed in sac1-26α, sac1-27α, sac1-28α, and sac1-29α mutants. Some 10–15% of total secretory invertase was found in the latent (i.e., intracellular) form in those sac1α mutants, as compared with 5% for the isogenic SAC1 strain. Second, thin section electron microscopy of these sac1α mutants did not reveal any of the gross ultrastructural phenotypes characteristic of other sec mutants. An exaggeration of intracellular membrane of unknown etiology was observed in only some 1–5% of the cells, and this behavior was consistent with the apparent secretory competence of these strains at 13°C.

A notable characteristic of the original sac1α mutants was the manifestation of gross defects in their pattern of chitin deposition at 13°C (Novick et al., 1989). To determine whether this phenotype was also exhibited by our sac1α mutants, we evaluated the distribution of cell wall chitin in sac1-26α and sac1-29α mutants. Isogenic strains CTY182 (SEC14, SAC1), CTY1-IA (sec14-1α, SAC1), CTY100 (sec14-1α, sac1-26α), and CTY109 (sec14-1α, sac1-29α) were grown to early logarithmic phase in YPD medium at 30°C. Subsequently, the cultures were shifted to 13°C for an incubation of 12 h, a period that essentially corresponded to a single generation time for these strains at that temperature in YPD. Cells were then fixed, stained with Calcofluor, and chitin staining was recorded as described above (see Materials and Methods). The results are shown in Fig. 3. Rather than depositing chitin exclusively to the bud neck, as was observed for the isogenic SEC14, SAC1 and sec14-1α, SAC1
strains, the \( sacl \) mutants exhibited a marked localization of chitin to heavy patches in the growing bud (Fig. 3). Such heavy patches of staining were also occasionally observed in mother cells. This aberrant chitin deposition was only observed when the \( sacl \) mutants were challenged with the 13°C condition. Normal staining was observed for \( sacl \) strains shifted to 30°C or 37°C. Moreover, these gross defects in chitin deposition did not seem to be the trivial result of challenging a conditional mutant with restrictive conditions. Strain CTY1-IA \( (sec14-1^w, \textit{SacI}) \), after shift to the restrictive temperature of 37°C for 2 h (i.e., one generation time), still displayed the wild-type pattern of chitin deposition. Thus, cell surface organization appeared to be specifically defective in these \( sacl \) mutants at 13°C.

Because the various \( sacl \) phenotypes, including the \( cs \) trait, correlate with defects in the assembly of the actin cytoskeleton in the original \( sacl \) mutants (Novick et al., 1989) we investigated whether the \( sacl \) mutants reported here also suffered from actin dysfunction at 13°C. Staining of polymeric actin structures with rhodamine-labeled phalloidin indicated that, as for the \( sacl \) mutants of Novick et al. (1989), a gross disorganization of the actin cytoskeleton was observed when our new \( sacl \) mutants were challenged with the 13°C block. This disorganization was manifested in two ways: (a) the filamentous actin network disappeared; and (b) cortical actin patches were no longer restricted to the bud but, rather, were uniformly distributed between buds and mother cells (not shown).

Another distinguishing feature of \( SAC1 \) is its unusual pattern of genetic interaction with \( act1-1^w \). Novick et al. (1989) reported that deletions of \( SAC1 \) resulted in a \( cs \) phenotype. The recessive nature and \( cs \) character of \( sacl \) thus suggested that suppression is by loss of \( SAClp \) function. However, deletion of \( SAC1 \) does not generate an \( act1-1^w \) suppressing allele. The basis of this puzzling genetic behavior is unknown (Novick et al., 1989). To test whether aspects of this unusual genetic behavior of \( SAC1 \) also extended to \( sec14-1^w \), we constructed a defined set of \( sacl \) disruption alleles in vitro. These disruptions were then recombined into \( SAC1 \) in vivo, confirmed to be correct by Southern analyses, and the phenotypic consequences with respect to viability, cold sensitivity, and suppression were determined (see Materials and Methods). The precise nature of the \( sacl \) disruption alleles is presented in Fig. 4, along with the phenotypic consequences of each.

In concordance with the conclusion of Novick et al. (1989), our analysis of the four \( sacl \) disruption alleles clearly demonstrated that \( SAC1 \) was not an essential gene. It was essential for growth of yeast at low temperatures, however, as the phenotype of haploid yeast bearing any of these four disruptions was a tight \( cs \) (Fig. 4). These results were observed regardless of whether the \( sacl \)-disrupted haploids were generated by meiotic means or by direct transformation of the \( sacl \) disruption allele. Furthermore, none of the four \( sacl \) disruption alleles elicited an efficient phenotypic suppression of \( sec14-1^w \) (Fig. 4). This was indicated by the \( ts \) phenotype of \( sec14-1^w \) strains that received the \( sacl \) disruptions via linear transformation. We noted, however, that this \( ts \) phenotype was often leakier than that observed for isogenic \( sec14-1^w \), \( SAC1 \) strains, perhaps indicative of a partial suppression. Nonetheless, our observation that the \( SAC1 \) disruption alleles did not approach the efficiency with which \( sacl \) alleles phenotypically suppressed \( sec14-1^w \) (see below) argued that suppression was not the result of elimination of \( SAClp \) function. Rather, these data were most simply reconciled with a model whereby suppression occurred by attenuation of \( SAClp \) activity. Although it remains unclear precisely how suppression might work, it was nevertheless significant that similar patterns of genetic interaction were observed with respect to suppression of defects in yeast secretory pathway and yeast actin function.

\( sacl \)-mediated Suppression of \( sec14-1^w \) Is Complete

In terms of growth rate on solid or liquid complex medium, all 14 of the new \( sacl \) alleles that we isolated appeared to effect an efficient suppression of \( sec14-1^w \). These mutations supported wild-type to near wild-type growth of \( sec14-1^w \) strains at 37°C, an absolutely restrictive temperature for the \( ts \) mutant. To obtain a more quantitative assessment of suppression efficiency, we measured the effect of \( sacl \) on invertase secretion in \( sec14-1^w \) strains challenged with the 37°C condition.

The isogenic strains CTY182 \( (SEC14) \), CTY1-IA \( (sec14-1^w) \), CTY100 \( (sec14-1^w, sacl-26^c) \), CTY101 \( (sec14-1^w, sacl-27^c) \), CTY108 \( (sec14-1^w, sacl-28^c) \), and CTY109 \( (sec14-1^w, sacl-29^c) \) were grown at 30°C in YP medium that was supplemented with glucose to 2% (see Table I for complete genotypes). The cells were induced for secretory invertase synthesis and imposition of the \( sec14-1^w \) block by shift of

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**Figure 3.** Chitin localization in wild-type and \( sacl \) mutant cells. Cells were grown at 30°C, shifted to 13°C as described in the text, and subsequently stained with Calcofluor. The relevant genotypes of the yeast strains analyzed are given along side the corresponding profiles.
Figure 4. Disruptions of SAC1. The precise structures of four sacl disruption alleles are given along with their associated haploid-specific phenotypes, their sacl-1<sup>+</sup> suppressor capabilities, and their allelic designations. An illustration of the wild-type SAC1 locus and its associated properties are also indicated at the top. The restriction site abbreviations and assumptions concerning the extent of the coding region are described in the legend to Figs. 1 and 2. The sacl-44::URA3 disruption was constructed by inserting a 1.2-kb Sal I fragment carrying URA3 into the Xho I site of SAC/. Disruptions of SAC1 at Bcl I (sacl-296::HIS3) and Bam HI (sacl-560::HIS3) were accomplished by inserting a 1.8-kb Bam HI fragment carrying HIS3 into these compatible half-sites. Disruption at Pvu II (sacl-467::HIS3) was performed by filling in the Bam HI half-sites of the HIS3 fragment and blunt-end ligating the fragment into the Pvu II site. For all these manipulations but the Pvu II disruption, the 2.4-kb Xba I fragment was maintained in pTZ18U (Mead et al., 1986) and liberated for linear transformation by digestion with Xba I. The Pvu II disruption was constructed by deleting an ~3-kb Sst I fragment from pCTY100 (see text and Fig. 3), leaving the SAC1 Pvu II site as a unique site in the plasmid. The disruption was then constructed as described above.

Table II. Invertase Secretion in Suppression Strains

| Strain     | Relevant genotype | Total invertase | Extracellular invertase |
|------------|-------------------|-----------------|-------------------------|
| CTY182     | sec14-1<sup>*</sup>, SAC1 | 305.4           | 306.2 (>98%)             |
| CTY1-1A    | sec14-1<sup>*</sup>, SAC1 | 483.7           | 95.7 (20%)               |
| CTY100     | sec14-1<sup>+</sup>, sacl-26<sup>a</sup> | 388.8           | 402.2 (>98%)             |
| CTY101     | sec14-1<sup>+</sup>, sacl-27<sup>a</sup> | 422.1           | 414.2 (98%)               |
| CTY108     | sec14-1<sup>+</sup>, sacl-28<sup>a</sup> | 507.5           | 488.8 (96%)               |
| CTY109     | sec14-1<sup>+</sup>, sacl-29<sup>a</sup> | 382.6           | 395.5 (>98%)             |

Yeast cells were grown at 30°C in YPD and shifted to 0.1% glucose YP medium for 30 min, and a subsequent shift to 37°C, respectively. After a 2-h incubation, extracellular and total invertase activities were determined for each strain (see Materials and Methods). As is evident from the representative data shown in Table II, the wild-type strain (CTY182) exhibited essentially all of its invertase activity at the cell surface. This was indicated by the fact that the measured values for extracellular and total invertase were equal. For the sec14-1<sup>+</sup> strain, however, only some 20% of the total invertase activity was extracellular. The remaining 80% was latent and therefore intracellularly disposed. Inspection of the invertase activity in the four sacl-<sup>a</sup> strains revealed a distribution that was comparable to that of the wild-type strain. Greater than 95% of the total invertase in these suppressor mutants was extracellular (Table II). We also noted that all six yeast strains exhibited approximately equal levels of total invertase activity, suggesting that direct comparisons between these strains were valid. Thus, suppression of sec14-1<sup>+</sup> by sacl-<sup>a</sup> mutations was essentially complete on the basis of growth and secretory competence at 37°C. Further evidence for efficient suppression was derived from electron microscopic visualization of thin sections obtained from sacl-<sup>a</sup>, sec14-1<sup>+</sup> yeast grown at 37°C.

These analyses indicated that the organelle exaggeration phenotype characteristic of sec14-1<sup>+</sup> strains at 37°C was alleviated (data not shown).

sac1<sup>a</sup> Can Suppress sec14 Disruption Alleles

The efficiency of suppression raised the issue of whether suppression was effected by a bypass mechanism or an interaction mode (for a discussion see Botstein and Maurer, 1982). These two mechanisms can be distinguished on the basis of...
whether suppression exhibits allele specificity or not. The bypass mechanism would be characterized by allele nonspecificity, whereas suppression by interaction might be expected to show allele specificity. The existence of only the one sec14-1 allele precluded a proper test of the allele specific nature of the suppression. However, since bypass suppressors should suppress all recessive alleles, we tested whether sac1 was capable of suppressing what are most likely sec14 null mutations.

We have previously described two SEC14 disruptions, sec14A1::HIS3 and sec14-129::HIS3, that constitute recessive lethal mutations (Bankaitis et al., 1989). The latter disruption was recombined into the sec14-1 allele of a set of four sec14-1::SEC14, sac1::SAC1 heterozygous diploids that represented the sac1-26°, sac1-27°, sac1-28°, and sac1-29° suppressor alleles (CTYD61, CTYD62, CTYD63, and CTYD64, respectively; see Table I). If sac1 could not suppress the relevant disruption, a 2:2 segregation of viable to nonviable spores should be observed with none of the viable spores exhibiting a His+ phenotype. The results of these crosses are shown in Table III. Although only the 2:2 segregation was observed for the four asci derived from CTYD61 that were analyzed, a departure from this pattern was noted for tetrads derived from CTYD62, CTYD63, and CTYD64. Asci exhibiting a 3:1 segregation of viable to nonviable spores were recovered from CTYD62, CTYD63, and CTYD64. Furthermore, an ascus exhibiting a 4:0 segregation was yielded by CTYD64. For each 3:1 ascus recovered, only one of the meiotic segregants was His+. In the case of the 4:0 ascus there were two His+ meiotic progeny. Each haploid His+ segregant also exhibited the cs phenotype diagnostic of the mutant sac1 allele. These data were consistent with the ability of at least the sac1-27°, sac1-28°, and sac1-29° alleles to suppress sec14-129::HIS3. This suppression was not considered to be complete, as the His+ segregants grew rather slowly (not shown), but significant as viability was restored.

To confirm that the His+ meiotic progeny has inherited sec14-129::HIS3, genomic DNA was prepared from representative segregants and their sister progeny and digested with Bam HI. The digests were fractionated on agarose gels, and subjected to Southern hybridization analysis (see Materials and Methods). As the data in Fig. 5 clearly show, the His+ segregants had inherited sec14-129::HIS3, whereas the His- sister progeny exhibited an unadulterated sec14 loci. This was indicated by a 3.8-kb SEC14 hybridizing species in the genomic digests of His+ segregant DNA, as opposed to the 2.0-kb species revealed in the genomic digests of His- sister DNA (see Fig. 5). Consistent genetic and biochemical results were also obtained with sec14A1::HIS3 (not shown).

Together, these data indicated that sac1 alleles could suppress sec14-129::HIS3 and sec14A1::HIS3, and suggested a bypass component to the suppression. As neither sec14-129::HIS3 nor sec14A1::HIS3 can be dosage compensated, these data made it unlikely that suppression could be mediated through elevation of sec14-1° gene product levels. We had considered the possibility of encountering such suppressors as these would have passed the original Ts+ selection. Northern analyses of SEC14 mRNA levels and measurements of relative SEC14p abundance in wild-type and sac1 yeast
confirmed that suppression was not exerted through dosage of Sec14p (data not shown). Thus, it seemed that at least certain sac1Δ alleles rendered the yeast cell independent of the usually essential SEC14p requirement. This independence apparently was not complete, however, as evidenced by the slow growth of the viable sec14Δ, sac1Δ mutants.

Range of Suppression

To determine whether the range of sac1Δ suppression extended beyond sec14Δ-1 and acl1Δ-1, we analyzed the phenotypic effect of sac1Δ on the other secΔ mutations that result in conditional defects at specific stages of the yeast secretory pathway. The sec6Δ allele was chosen because it was generated in response to actin dysfunction and was considered to be naive with respect to defects in the secretory pathway. Standard genetic methods were employed to construct the appropriate secΔ, sac1Δ haploid double mutants. These were subsequently tested for growth at 25°C, 30°C, 33.5°C, and 37°C. The double mutant growth patterns were then compared with those obtained for the parental secΔ strains.

As is apparent from the data in Table IV, sac1Δ exhibited a significant suppression of only two additional sec mutations other than the sec14Δ-1 Golgi block. These were the sec6Δ-4 and sec9Δ-4 secretory vesicle blocks. Although suppression of sec14Δ-1 was clearly efficient (see above), the effect on sec9Δ-4 and sec6Δ-4 appeared to be more modest. Suppression of the latter was observed just at 33.5°C, a minimally restrictive temperature. From these data we concluded that sac1Δ did not exhibit a general suppressor capability with regard to yeast secretory defects. Furthermore, the limited spectrum of sac1Δ-mediated suppression, coupled with the ability of sac1 to suppress sec14 disruptions, also suggested a specificity for suppression that was not immediately consistent with a model by which suppression was the result of some general stabilization of thermolabile proteins. This analysis did, nevertheless, extend the spectrum of sac1 suppression to four members; acl1Δ, sec14Δ-1, sec9Δ-4, and sec6Δ-4. Although it is not known which Golgi compartment is defective in the sec14Δ-1 mutant, we found it noteworthy that the other suppressible mutations block late stages of the yeast secretory pathway. The actin, sec9Δ-4, and sec6Δ-4 mutations affect post-Golgi events (Novick et al., 1981; Novick and Botstein, 1985).

During the course of these double mutant analyses, we made the rather unexpected observation that sac1Δ appeared to exhibit negative genetic interactions with several sec mutations. Two striking examples of such an antagonism were sec13Δ-1 and sec20Δ-1 (Table IV). In the case of sec13Δ-1, a high degree of spore inviability resulted from crosses to sec6Δ-4. The pattern of inviability was consistent with the sec13Δ-1, sac1Δ double mutant combination being lethal. The case of sec20Δ-1 was only slightly less extreme. In this instance, the sec20Δ-1, sac1Δ double mutants were very sick and grew only slowly at 25°C. Several milder examples of antagonism were also observed. The sec17Δ-1, sec8Δ-1, sec21Δ-1, and sec23Δ-1 yeast were noticeably more ts in a sac1Δ-6Δ genetic background than in a SAC1 background (Table IV). We noted that these six antagonized sec mutations result in a block in protein transport from the endoplasmic reticulum to the Golgi (Novick et al., 1981). Thus, sac1 mutations exhibited not only powerful compensatory interactions but also clear negative genetic interactions with yeast secretory defects.

**Discussion**

The ability of SAC1p to concomitantly influence both yeast Golgi function and the state of the actin cytoskeleton is intriguing in that the yeast secretory pathway exhibits a high degree of polarity directed towards the bud. This polarity is essential for proper yeast cell growth, and it is believed to be achieved by a coupling of the late stages of the yeast secretory pathway to the actin cytoskeleton. The suppressor properties of sac1Δ mutations, and the associated cs defects in actin assembly, lead us to speculate that the SAC1p may represent one aspect of a mechanism whereby such an essential coupling is regulated. Perhaps by modulating both secretory and cytoskeletal activities the SAC1p may coordinate actin assembly in response to the flow of secretory material from the Golgi complex. Because sac1Δ disruptions manifested themselves in a cs phenotype (Fig. 4), such a coordinating activity would only be essential at low temperatures. One possible reason for such a selective requirement might be that redundant activities operate at higher temperatures. We also note that a regulatory role for SAC1p would satisfactorily account for the apparent secretory competence of sac1Δ mutants at 13°C. That is, secretion per se is not defective, but its spatial regulation may be. This notion is consistent with the suppressor properties of sac1Δ mutations and with the defects in chitin deposition in these mutants at 13°C (Fig. 3).

The range of sac1Δ-mediated suppression was limited. Of

### Table IV. Suppression of secΔ by sac1Δ-6Δ

| secΔ, SAC1 | secΔ, sac1Δ-6Δ |
|------------|---------------|
| 25°C       | 30°C          | 33.5°C       | 37°C        |
| sec1-1     | +             | +             | +           | +           |+/−/−/− |
| sec2-41    | +             | +             | +           | +           |+/−/−/− |
| sec3-2     | +             | +             | +           | +           |−/−/−/− |
| sec4-8     | +             | +             | +           | +           |+−/−/− |
| sec5-24    | +             | +             | +           | +           |−/−/−/− |
| sec6-4*    | +             | +             | +           | +           |+−/−/− |
| sec7-1     | +             | +             | +           | +           |+−/−/− |
| sec8-9     | +             | +             | +           | +           |+−/−/− |
| sec9-4*    | +             | +             | +           | +           |+−/−/− |
| sec10-2    | +             | +             | +           | +           |+−/−/− |
| sec12-4    | +             | +             | +           | +           |−/−/−/− |
| sec13-11   | +             | +             | +           | +           |+−/−/− |
| sec14-1*   | +             | +             | +           | +           |+−/−/− |
| sec15-1    | +             | +             | +           | +           |+−/−/− |
| sec16-2    | +             | +             | +           | +           |+−/−/− |
| sec17-11   | +             | +             | +           | +           |+−/−/− |
| sec18-11   | +             | +             | +           | +           |+−/−/− |
| sec19-1    | +             | +             | +           | +           |+−/−/− |
| sec20-11   | +             | +             | +           | +           |−/−/−/− |
| sec21-11   | +             | +             | +           | +           |+−/−/− |
| sec22-3    | +             | +             | +           | +           |+−/−/− |
| sec23-11   | +             | +             | +           | +           |+−/−/− |

The growth properties of secΔ, SAC1 strains were compared with those of corresponding secΔ, sac1Δ-6Δ double mutants at the indicated temperatures. The secΔ, sac1Δ-6Δ strains were constructed by standard meiotic crosses where mating, sporulation, dissection, and germination were performed at 25°C.

1 Negative genetic interaction observed between secΔ and sac1Δ-6Δ.
2 Synthetic lethality observed in the secΔ, sac1Δ-6Δ configuration.
3 Significant suppression of secΔ by sac1Δ-6Δ.
the 23 sec mutants complementation groups that define gene products acting at specific stages of the secretory pathway (Novick et al., 1980), only the sec4-1, sec9-4, and sec6-4 alleles were suppressed by the sac1-6 mutation (Table IV). Moreover, this pattern of genetic interaction suggested a relationship between SEC14p, SEC6p, SEC9p, and actin. We do not presently believe that such a relationship necessarily involves direct interaction between these gene products. We have constructed sec4-1, sec6-4, and sec4-1, act1-1 haploid strains and failed to observe any indication of synthetic lethality, a genetic criterion for the identification of interacting proteins (Sturtevant, 1956; Bankaitis and Bassford, 1985; Novick et al., 1989). The act1-1, sec6-4, and act1-1/sec9-4 double mutant combinations have also been reported not to exhibit a synthetic lethality (Salminen and Novick, 1987). Although the precise nature of the relationship between these gene products remains unclear, we note that sec6-4, sec9-4, and act1-1 mutants are defective in post-Golgi events. By association, this also implies a late involvement for SEC14p in the yeast secretory pathway, perhaps at the level of protein exit from the yeast Golgi complex. We note that such an “exit from Golgi” stage would provide a reasonable point for coordinating secretory pathway activity with actin cytoskeleton function.

The synthetic lethality associated with the pairing of sac1-6 with either sec13-1 or sec20-1 suggests a broader pattern of genetic interaction for sac1 alleles and sec mutations than what was indicated by suppression alone (Table IV). This view was supported by our finding that sac1-6 also antagonized sec17-1, sec8-1, sec2f-1, and sec23-1 (Table IV). These negatively affected alleles encode products that are involved in protein transport from the ER to the yeast Golgi (Novick et al., 1980), and thus help mediate the early stages of the secretory pathway. These data, coupled with the suppression analyses, suggested that either the SAC1p influenced both early and late secretory events, or that the six antagonized gene products have execution points in the early and the late stages of the secretory pathway. A role for SAC1p in modulating only late events (as suggested by the suppression data; Table IV) could still be consistent with all of the data if the latter scenario were true. We note that assignment of the SEC13, SEC17, SEC18, SEC20, SEC21, and SEC23 gene products to early stages of the secretory pathway is based upon an analysis that will reveal only the first execution point (Novick et al., 1980). It has recently been shown that several early acting SEC gene products, including two that exhibit negative genetic interactions with sac1-6 (i.e., SEC18 and SEC23), are also required for fluid phase endocytosis in yeast (Riezman, 1985). This is suggestive of a requirement for these two gene products at both early and late stages of the secretory pathway. Further evidence pointing to a more global participation of the SEC18 gene product in the secretory pathway has recently been obtained. Wilson et al. (1989) have shown that the yeast SEC18p is a structural and functional homologue of the mammalian N-ethylmaleimidesensitive factor (NSF) that is required for intercisternal transport of proteins through the Golgi complex. Moreover, Beckers et al. (1989) have found NSF to play a role in ER-derived vesicle fusion to the Golgi complex, whereas Diaz et al. (1989) have demonstrated an NSF requirement for endocytic vesicle fusion in a cell-free system obtained from macrophages. In the context of SEC18, the synthetic lethality and negative interaction data that we have presented here suggest that, like SEC18p, the SEC13, SEC17, SEC20, and SEC21 gene products may also display multiple execution points in the exocytic pathway.

In conclusion, we have presented genetic evidence that SAC1p function is relevant to both the activity of the yeast secretory pathway and the actin cytoskeleton. On this basis, we have suggested that the SAC1p may be involved in the coordination of the activities of these two systems. Such a coherent regulation is essential for proper growth of the yeast cell. We believe that further genetic and biochemical analyses of SEC14p, SAC1p, and associated activities will prove informative with respect to how essential secretory and cytoskeletal functions communicate, and the nature of the cellular machinery that governs polarized growth of cell surfaces.

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