RSR1, a ras-like gene homologous to Krev-1 (smg21A/rap1A): role in the development of cell polarity and interactions with the Ras pathway in Saccharomyces cerevisiae.

R Ruggieri, A Bender, Y Matsui, S Powers, Y Takai, J R Pringle and K Matsumoto

---

Updated information and services can be found at:
http://mcb.asm.org/content/12/2/758

These include:

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

RSR1, a *ras*-Like Gene Homologous to K*rev*-1 (*smg21A/rap1A*): Role in the Development of Cell Polarity and Interactions with the Ras Pathway in *Saccharomyces cerevisiae*

ROSAMARIA RUGGIERI,[1]† ALAN BENDER,[2]‡ YASUSHI MATSUI,[3] SCOTT POWERS,[4] YOSHIMI TAKAI,[5]
JOHN R. PRINGLE,[2]§ AND KUNIHIRO MATSUMOTO[1]∥*

*DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304[1]; Department of Biology,
The University of Michigan, Ann Arbor, Michigan 48109[2]; Department of Biology, University of Tokyo,
Tokyo 113, Japan[3]; Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway,
New Jersey 08854[4]; and Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan[5]*

The *Saccharomyces cerevisiae ras*-like gene *RSR1* is particularly closely related to the mammalian gene K*rev*-1 (also known as *smg21A* and *rap1A*). *RSR1* was originally isolated as a multicopy suppressor of a *cdc24* mutation, which causes an inability to bud or establish cell polarity. Deletion of *RSR1* itself does not affect growth but causes a randomization of bud position. We have now constructed mutant alleles of *RSR1* encoding proteins with substitutions of Val for Gly at position 12 (analogous to constitutively activated Ras proteins) or Asn for Lys at position 16 (analogous to a dominant-negative Ras protein). *rsr1*$^{Val-12}$ could not restore a normal budding pattern to an *rsr1* deletion strain but could suppress a *cdc24* mutation when overexpressed. *rsr1*$^{Asn-16}$ could randomize the budding pattern of a wild-type strain even in low copy number but was not lethal even in high copy number. These and other results suggest that Rsr1p functions only in bud site selection and not in subsequent events of polarity establishment and bud formation, that this function involves a cycling between GTP-bound and GDP-bound forms of the protein, and that the suppression of *cdc24* involves direct interaction between Rsr1p[GTP] and Cdc24p. Functional homology between Rsr1p and K*rev*-1 p21 was suggested by the observations that expression of the latter protein in yeast cells could both suppress a *cdc24* mutation and randomize the budding pattern of wild-type cells. As K*rev*-1 overexpression can suppress *ras*-induced transformation of mammalian cells, we looked for effects of *RSR1* on the *S. cerevisiae* Ras pathway. Although no suppression of the activated RAS2^{Val-19} allele was observed, overexpression of *rsr1*$^{Val-12}$ suppressed the lethality of strains lacking *RAS* gene function, apparently through a direct activation of adenylyl cyclase. This interaction of Rsr1p with the effector of Ras in *S. cerevisiae* suggests that K*rev*-1 may revert *ras*-induced transformation of mammalian cells by affecting the interaction of *ras* p21 with its effector.

Ras proteins are important regulators of cell proliferation and, when deregulated, can cause neoplastic transformation. They bind and hydrolyze GTP, working as molecular switches in transducing growth or differentiation signals (4, 19). The oncogenic transformation induced by activated K-*ras* can be suppressed by overexpression of another GTP-binding protein, the product of the K*rev*-1 gene (also known as *smg21A* and *rap1A*), whose sequence is ~53% identical to that of the K-*ras* product (31, 33, 42). This sequence similarity includes an exact conservation of the putative effector domain (amino acids 32 to 42 [4, 24, 51]). The mechanism by which K*rev*-1 suppresses *ras*-induced transformation is unclear and could involve either direct interference with the effector function(s) stimulated by *ras* p21 or activation of an alternative pathway that leads to growth inhibition. It has been reported that K*rev*-1 p21 binds tightly to *ras* GTPase-activating protein (GAP) in vitro (18,

24); although the GTPase activity of K*rev*-1 p21 is not activated by *ras* GAP, K*rev*-1 p21 acts effectively as a competitive inhibitor of the stimulation of *ras* GTPase activity by *ras* GAP (18). These observations suggest that K*rev*-1 antagonizes *ras*-induced transformation by sequestering *ras* GAP or some other protein(s) that is needed for Ras function.

Because of the extent of functional conservation between yeast and mammalian cells, *Saccharomyces cerevisiae* has been an invaluable tool in the analysis of Ras function. In this yeast, the *RAS1* and *RAS2* gene products play a central role in growth control and function, at least in part, by stimulation of adenylyl cyclase (19, 60). The *CDC25* and *IRA* gene products are involved in the regulation of Ras1p and Ras2p activity. Cdc25p is a positive element that promotes formation of the active Ras-GTP complex, probably by acting as a nucleotide exchange factor (11, 16, 28, 34, 46). Ira1p and Ira2p are negative elements that promote the formation of the Ras-GDP complex, probably by stimulating *ras* GTPase activity (54, 56–58). The Ira proteins share significant amino acid sequence similarity with mammalian *ras* GAP in its catalytic domain, and *ras* GAP can complement the loss of *IRA1* and *IRA2* (3, 57).

In the past few years, it has become clear that eukaryotic cells contain a large number of Ras-related proteins, which apparently serve a wide variety of roles in cell physiology (8, 9, 21). One recent addition to this Ras superfamily is the

---

* Corresponding author.
† Present address: Department of Molecular Biology, Cetus Corporation, Emeryville, CA 94608.
‡ Present address: Department of Biology, Indiana University, Bloomington, IN 47405.
§ Present address: Department of Biology, CB 3280 Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280.
∥ Present address: Department of Molecular Biology, School of Science, Nagoya University, Nagoya, Japan, 464-01.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source or reference
Y308	*MATα ura3 leu2*	Segregant from Y246 (5)
SY1229	*MATa ura3 leu2 his3*	6
Y301	*MATα rsr1*::URA3 *ura3 leu2 his3*	5
Y419	*MATα rsr1*::*ura3 ura3 leu2 his3*	Y301 rendered Ura⁻ by growth on 5-fluoroorotic acid
Y147	*MATa cdc24-4 ura3 leu2 his3*	5
TK161-R2V	*MATa RAS2^{Val-19} ura3 leu2 trp1 his3 ade8 can1*	60
T50-3A	*MATa cyr1-2 ura3 leu2 trp1 his3*	29
RS60-15B	*MATα RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 ade2 ade8*	Segregant from RS16-4C × STS13 (44)
KMY208-3C	*MATα cdc25-1 ura3 leu2 trp1 ade2*	Derived from strain 321 (23)
TT1A-1	*MATα cdc25*::URA3 *ura3 leu2 trp1 his3 ade8* [p*CDC25*(*TRP1*)-1]	11
KMY401-4D	*MATa ira1*::LEU2 *RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 ade2 ade8*	Derived by several crosses from RS60-15B and KT7 (56)
KT62-2D	*MATα ira2*::HIS3 *ura3 leu2 trp1 his3*	K. Tanaka; derived from KT27 (58)
RRY8-5B	*MATα ira1*::LEU2 *RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 ade2*	Segregant from KMY401-4D × KT62-2D
RRY8-14B	*MATα ira2*::HIS3 *RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 ade2*	Segregant from KMY401-4D × KT62-2D
RRY8-4A	*MATα ira1*::LEU2 *ira2*::HIS3 *RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 ade8*	Segregant from KMY401-4D × KT62-2D
KMY45-2D	*MATa ras1*::URA3 *ura3 leu2 trp1 his3 ade8*	K. Matsumoto
KMY401-5A	*MATα RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 lys2 ade2*	As for KMY401-4D
RRY5-29B	*MATa ras1*::URA3 *RAS2$^{Val-19,Ala-22}$ ura3 leu2 trp1 his3 lys2*	Segregant from KMY45-2D × KMY401-5A
KMY45-2A	*MATa ras1*::HIS3 *ura3 leu2 trp1 his3 ade8*	K. Matsumoto
KMY46-4Bp	*MATα ras2*::URA3 *ura3 leu2 trp1 his3 ade8* [YEp(rsr1^{val12})]	Transformant of KMY46-4B (K. Matsumoto) with YEp(rsr1^{val12})
RRY9-19B	*MATa ura3 leu2 trp1 his3 ade8*	Segregant from KMY45-2A × KMY46-4Bp
RRY9-2D	*MATα ras1*::HIS3 *ras2*::URA3 *ura3 leu2 trp1 his3 ade8* [YEp(rsr1$^{val\ 12}$)]	Segregant from KMY45-2A × KMY46-4Bp
RRY9-26A	*MATa ras1*::HIS3 *ras2*::URA3 *ura3 leu2 trp1 his3 ade8* [YEp(rsr1^{val12})]	Segregant from KMY45-2A × KMY46-4Bp

product of the *S. cerevisiae RSR1* gene, which was originally identified as a multicopy suppressor of a temperature-sensitive *cdc24* mutation (5). The *CDC24* product is involved in the morphogenetic events of the *S. cerevisiae* cell division cycle, including the selection of a nonrandom budding site and the subsequent polarization of secretion and localization of cell wall deposition to the bud (53). Deletion of *RSR1* itself has no effect on growth rate but randomizes the selection of budding sites, suggesting that Rsr1p may be necessary for selection of the normal budding site but not for subsequent events in the establishment of cell polarity and bud formation (5, 15). Among the members of the Ras superfamily, Rsr1p is most similar to the mammalian K*rev*-1 gene product, with which it shares 65% identity over the first 125 amino acids and 57% identity over the entire length (184 amino acids) of K*rev*-1 p21 (5), including a complete identity with the putative effector-binding domains of both *ras* p21 and K*rev*-1 p21.

In this report, we explore further the role of *RSR1* in morphogenesis through the construction and analysis of mutant alleles designed to be analogous to *ras* alleles that result in constitutive activation or a dominant-negative effect. In addition, we characterize further the similarity of Rsr1p to K*rev*-1 p21. In this respect, we show that the two proteins share some functional homology and that overexpression of a mutant *rsr1* allele affects the yeast Rasmediated starvation response pathway by interacting with adenylyl cyclase, the Ras effector in this organism.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. The *S. cerevisiae* strains used in this study are described in Table 1, and the plasmids used are described in Table 2. Yeast cells were grown on standard rich (YPD or YM-P), defined minimal (SD), defined complete (SC), or sporulation medium (35, 50) at various temperatures as noted below. Selective media for isolation of transformants and for determination of auxotrophic requirements were SC lacking the appropriate requirement. SD+His medium was SD containing 25 μg of histidine per ml; SD+His+Sorb medium was SD+His containing 1 M sorbitol.

Recombinant DNA and genetic procedures. Except as noted, standard procedures were used for recombinant DNA manipulations, propagation of plasmids in *Escherichia coli*, yeast transformations, and yeast genetics (26, 48, 50). To create the *rsr1*$^{Val-12}$ and *rsr1*$^{Asn-16}$ alleles, a 1.6-kb *Sac*I-*Bgl*II fragment containing all of *RSR1* (5) was inserted into *Sac*I-*Bam*HI-cut M13mp19 (63) to create phage ABM5. Point mutations were then created in ABM5 by using synthetic oligonucleotides and the Bio-Rad (Richmond, Calif.) Muta-Gene kit. The oligonucleotides used were 5′-G GGT GCT GTT GGT GTC G-3′, which should change *RSR1* codon 12 (GGT, Gly) to GTT (Val), and 5′-GTC GGT AAT TCC TGC TT-3′, which should change *RSR1* codon 16 (AAA, Lys) to AAT (Asn). By using DNA sequencing (49), two independent DNAs containing each of the mutations were identified.

TABLE 2. Plasmids

Plasmid	Characteristics	Source or reference
YCp50	URA3, ARS1, CEN4 (low copy number)	47
YEp13	LEU2, 2μm ARS^a (high copy number)	10
pSL113	LEU2, 2μm ARS (rearrangement of YEp13; high copy number)	5
YEp24	URA3, 2μm ARS (high copy number)	7
pKT10	URA3, 2μm ARS, TDH3 (glyceraldehyde-3-phosphate dehydrogenase) promoter (high copy number)	K. Tanaka (25, 38, 55)
pPB117	RSR1 in YEp24	Original isolate from YEp24 library (5)
YCp(RSR1)	RSR1 in YCp50	This study (see text)
YEp(RSR1)	RSR1 in pSL113	This study (see text)
YCp(rsr1^{Val12})	rsr1^{Val-12} in YCp50	This study (see text)
YEp(rsr1^{Val12})	rsr1^{Val-12} in pSL113	This study (see text)
pPB410	rsr1^{Val-12} in YEp24	This study (see text)
YCp(rsr1^{asn16})	rsr1^{Asn-16} in YCp50	This study (see text)
YEp(rsr1^{asn16})	rsr1^{Asn-16} in pSL113	This study (see text)
pKTsmg21A	EcoRI fragment containing bovine smg21A cDNA (31) inserted at the EcoRI site of pKT10 (and hence under control of the TDH3 promoter)	This study
pKTCsmg21	As for pKTsmg21A, except that most smg21A 3' noncoding sequences (everything 3' to the BglII site [31]) have been deleted	This study
YCpADH-RAS1	YCp50 containing RAS1 fused to the ADH1 (alcohol dehydrogenase) promoter	20, 37
YCpADH-RAS2	YCp50 containing RAS2 fused to the ADH1 promoter	20, 37
YCpADH-RAS2[Ala-18, Val-19]	YCp50 containing RAS2$^{Ala-18,Val-19}$ fused to the ADH1 promoter	37
AAH5-Ha-ras	LEU2, 2μm ARS, wild-type human ras gene fused to the ADH1 promoter (high copy number)	17, 20, 37
AAH5-Ha[Val-12, Thr-59]-ras	Like AAH5-HA-ras except mutant human ras gene	20, 37, 52
YEpRAS2-1	RAS2, LEU2, 2μm ARS (high copy number)	43
pRAS2^{val19}	RAS2^{Val-19}, LEU2, 2 μm ARS (high copy number)	11
YEpRHO1	RHO1, URA3, 2μm ARS (high copy number)	pWT of reference 36
pCDC25 (TRP1)-1	CDC25, TRP1, ARS1	11
YCpN1	TRP1, ARS1, CEN3 (low copy number)	41
YCpRAS2^{val19}	RAS2^{Val-19}, URA3, TRP1, ARS1, CEN4 (low copy number)	K. Tanaka
YEpTPK1	TPK1, LEU2, 2μm ARS (high copy number)	59

^{a} ARS, autonomously replicating sequence.

The mutant alleles were then cloned into YCp50, pSL113, and YEp24 by cutting the mutated ABM5 with BglII (0.55 kb away from the polylinker) and with SalI (in the polylinker) and in erting the resulting fragments into BamHI-SalI-cut YCp50, pSL113, or YEp24. Plasmid YCp(RSR1) was constructed similarly, using YCp50 and nonmutagenized ABM5. To construct plasmid YEp(RSR1), nonmutagenized ABM5 was cut with BglII and HindIII (in the polylinker), and the resulting fragment was inserted into BamHI-HindIII-cut pSL113.

Morphological observations. To visualize bud scars, cells were stained with Calcofluor and observed by fluorescence microscopy as described previously (45).

cAMP assay. Glucose-induced cyclic AMP (cAMP) formation was determined in stationary-phase cells as described previously (40), using the cAMP determination kit (Amersham, Arlington Heights, Ill.).

RESULTS

Functional characterization of rsr1 mutants. Rsr1p appears to be necessary for the normal selection of nonrandom budding sites but not for subsequent essential events in polarity establishment and bud formation (see the introduction). However, it is also possible that the nonlethality of the rsr1 deletion reflects functional redundancy of Rsr1p. To investigate this possibility and to explore further the role of Rsr1p in bud site selection, we used oligonucleotide-directed mutagenesis to create two rsr1 alleles that we thought might

have dominant-negative effects. In one mutant, glycine 12 was replaced by valine, producing a mutant Rsr1p analogous to the constitutively activated products of the human ras^{Val-12} and S. cerevisiae RAS2^{Val-19} alleles (4, 30). In the second mutant, lysine 16 was replaced by asparagine, producing a mutant Rsr1p analogous to Ha-ras p21^{Asn-16}, a protein that binds guanine nucleotides ~100-fold less well than does the wild type and has dominant-lethal effects when expressed in yeast cells (52).

When either mutant allele was introduced into the rsr1 deletion strain Y419 (Table 1) on a low-copy-number plasmid [YCp(rsr1^{val12}) or YCp(rsr1^{asn16}); Table 2], the transformants grew well but still displayed the randomized budding pattern characteristic of strains lacking Rsr1p (Fig. 1C and data not shown). In contrast, Y419 cells harboring a similar plasmid containing the wild-type RSR1 gene [YCp(RSR1)] displayed the normal unipolar budding pattern of haploid cells (Fig. 1A and B). When YCp(rsr1^{val12}) or YCp(rsr1^{asn16}) was introduced into the wild-type strain Y308, the transformants appeared to grow normally but displayed a randomized budding pattern (Fig. 1D and E); transformation of Y308 with YCp(RSR1) or YEp(RSR1) produced no such effect (data not shown). Introduction of either mutant allele at high copy number [strain Y301 transformed with plasmid YEp(rsr1^{val12}) or strain SY1229 transformed with plasmid YEp(rsr1^{asn16})] produced essentially the same phenotype: the cells grew well at 12, 30, and 37°C, but the budding pattern was randomized. These results indicate that neither
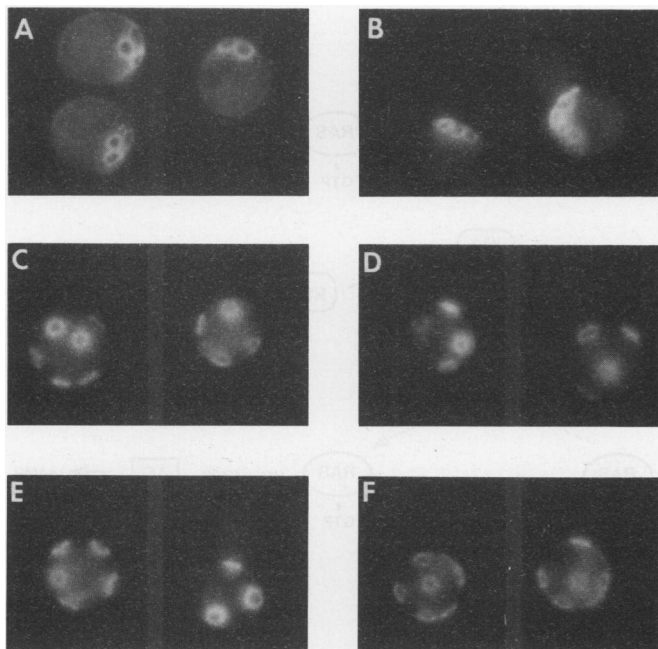
FIG. 1. Budding patterns in cells of different genotypes. Cells were grown at 23°C and observed by fluorescence microscopy after staining with Calcofluor. (A) Wild-type strain Y308 harboring control plasmid pKT10; (B) *rsr1* deletion strain Y419 harboring plasmid YCp(RSR1); (C) strain Y419 harboring plasmid YCp(rsr1^{val12}); (D) strain Y308 harboring plasmid YCp(rsr1^{val12}); (E) strain Y308 harboring plasmid YCp(rsr1^{asn16}); (F) strain Y308 harboring plasmid pKTC*smg*21. Strains and plasmids are described in Tables 1 and 2.
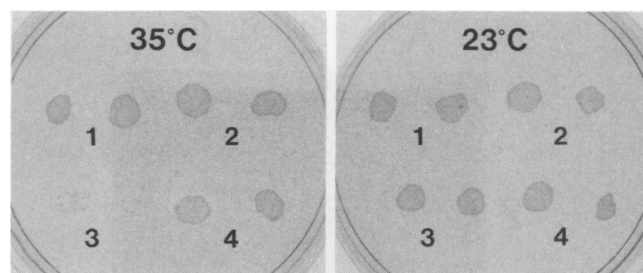

FIG. 2. Suppression of temperature sensitivity in a *cdc24* strain by wild-type and mutant *RSR1*. Strain Y147 was transformed with pSL113 or with YEp(rsr1^{asn16}). Each of the two resulting strains was transformed with pPB117 or with pPB410. For each of the four resulting strains, two independent transformants were grown to saturation in SD+His at 23°C, diluted 1:10 with SD, and spotted onto duplicate SD+His+Sorb plates. One plate was incubated at 23°C for 3 days, while the other was incubated at 35°C for 2 days. The plasmids present are (1) pPB117 and pSL113, (2) pPB410 and pSL113, (3) pPB117 and YEp(rsr1^{asn16}) and (4) pPB410 and YEp(rsr1^{asn16}). Plasmids are described in Table 2.

rsr1^{Val-12} nor *rsr1*$^{Asn-16}$ can supply Rsr1p function for normal bud site selection; indeed, the mutant proteins interfere with the function of the normal Rsr1p. Although this interference is observed even at low copy numbers, there is no pronounced effect on viability or growth rate even when the mutant alleles are present at high copy numbers.

We also tested the ability of *rsr1*$^{Val-12}$ and *rsr1*$^{Asn-16}$ to suppress a *cdc24*(Ts) mutation. When introduced into strain Y147, plasmid YEp(rsr1^{val12}) or pPB410 produced effective suppression (Fig. 2 and data not shown). In contrast, YCp(rsr1^{val12}), YCp(rsr1^{asn16}), and YEp(rsr1^{asn16}) all failed to suppress effectively when introduced into the same strain (data not shown). Indeed, *rsr1*$^{Asn-16}$ interfered with the suppression of *cdc24* by *RSR1*; that is, Y147 cells containing both YEp(rsr1^{asn16}) and pPB117 failed to grow at 35°C even in the presence of 1 M sorbitol (Fig. 2). In contrast, *rsr1*$^{Asn-16}$ did not interfere with the suppression of *cdc24* by *rsr1*$^{Val-12}$: Y147 cells containing both YEp(rsr1^{asn16}) and pPB410 could grow at 35°C (Fig. 2).

Functional homology of *RSR1* and K-*rev*-1. To determine whether the similarity in amino acid sequence between Rsr1p and K*rev*-1 p21 is associated with functional homology, we first examined whether expression of K*rev*-1 p21 in yeast cells could suppress the randomized budding pattern of the *rsr1* deletion strain. Transformation of strain Y419 with plasmid pKT*smg*21A produced no detectable correction of the abnormal budding pattern (data not shown), indicating that K*rev*-1 p21 cannot supply normal Rsr1p function. However, introduction of pKT*smg*21A or the related plasmid pKTC*smg*21A into the wild-type strain Y308 produced a randomization of the budding pattern (Fig. 1F and data not shown). This is not a general effect of overexpressing

ras-like genes in yeast cells, as no such effect was seen when Y308 was transformed with plasmid YCpADH-*RAS1*, YCpADH-*RAS2*, or YCpADH-*RAS2*[Ala-18, Val-19], while only a slight effect on budding pattern (occasional cells showing apparent bipolar budding) was seen with plasmid AAH5-Ha-*ras*. These results suggest that K*rev*-1 p21 can interact with at least some of the proteins with which Rsr1p normally interacts in the pathway that determines bud position.

This conclusion was supported by the ability of K*rev*-1 to suppress a *cdc24*(Ts) mutation. As in the case of *RSR1* itself, the suppression was effective on YPD medium at 35°C (Fig. 3A) but not at 37°C (Fig. 3B). As with the effect on bud position, this effect of K*rev*-1 was not a general property of *ras*-like genes, as plasmids YCpADH-*RAS1*, YCpADH-*RAS2*, YCpADH-*RAS2*[Ala-18, Val-19], YEp*RAS2*-1, pRAS2^{val19}, AAH5-Ha-*ras*, AAH5-Ha[Val-12,Thr-59]-*ras*, and YEp*RHO1* had little or no effect on the temperature sensitivity of the *cdc24* strain.

Suppression by *RSR1* of mutations that inactivate the yeast Ras pathway. Overexpression of K*rev*-1 can suppress trans-
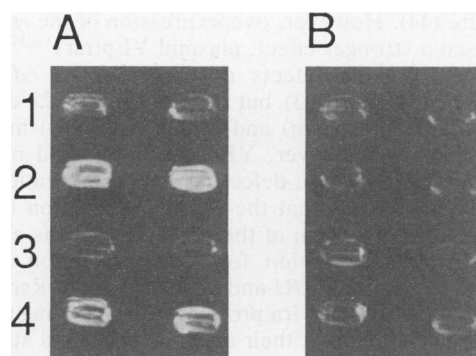

FIG. 3. Suppression of temperature sensitivity in a *cdc24* strain by K*rev*-1. Strain Y147 was transformed with various plasmids, and cells were patched onto YPD medium in duplicate and grown for 3 days at 35°C (A) or 37°C (B). Plasmids tested were (1) YEp24, (2) pPB117, (3) pKT10, and (4) pKT*smg*21A. Plasmids are described in Table 2.
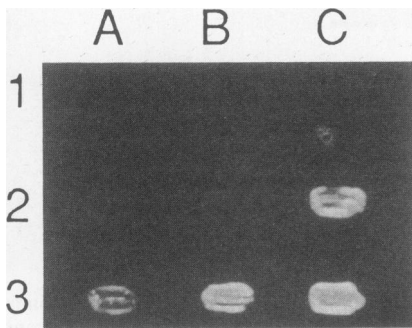
FIG. 4. Suppression of mutations of the Ras pathway by *RSR1* and *rsr1*$^{Val-12}$. Strains T50-3A [*cyr1*(Ts); row 1], RS60-15B [*RAS2*(Ts); row 2], and KMY208-3C [*cdc25*(Ts); row 3] were transformed with plasmids YEp13 (A), YEp(RSR1) (B), and YEp(rsr1^{val12}) (C) and tested for growth at 35°C as described in the legend to Fig. 3. Strain T50-3A was transformed only with plasmid YEp(rsr1^{val12}).

FIG. 5. Models for suppression of a *cdc25* defect by *rsr1*$^{Val-12}$. Solid boxes and arrows indicate active proteins and processes; hatched boxes and arrows indicate defective proteins and processes. See text for additional explanation. Similar models could be considered for the suppression of *RAS2*(Ts) by *rsr1*$^{Val-12}$; suppression through interaction with the Ira proteins (A) would be mediated by Ras1p.

formation caused by activated *ras* alleles in mammalian cells. As *RSR1* seems to have at least some functional homology to Krev-1, we tested whether overexpression of *RSR1* could suppress the phenotypes caused by the activated *RAS2*$^{Val-19}$ allele, such as sensitivity to heat shock and nitrogen starvation, failure to arrest in the G_1 phase of the cell cycle, and failure to sporulate (30, 60). However, neither plasmid YEp(RSR1) nor plasmid YEp(rsr1^{val12}) was able to suppress the phenotypes due to *RAS2*$^{Val-19}$ when introduced into strain TK161-R2V (data not shown). On the other hand, overexpression of *RSR1* did suppress a *cdc25*(Ts) mutation, which causes a defect in the activation of Ras at high temperatures (Fig. 4A and B, row 3). To test whether *RSR1* could suppress a complete loss of *CDC25* function, we introduced plasmid YEp(RSR1) into *cdc25*::*URA3* cells that carried the wild-type *CDC25* gene on a *TRP1*-containing plasmid (strain TT1A-1; Table 1). We then examined whether the *RSR1* plasmid could confer viability to these cells in the absence of the *CDC25* plasmid. However, when growth in rich medium was followed by replica plating to selective media, no Leu$^+$ Trp$^-$ colonies were observed, indicating that overexpression of *RSR1* could not suppress a deletion of *CDC25*. Overexpression of *RSR1* also failed to suppress a *RAS2*(Ts) allele (Fig. 4B, row 2), which is a dominant-negative allele believed to sequester Cdc25p while being unable to activate adenylyl cyclase at the restrictive temperature (44). However, overexpression of the *rsr1*$^{Val-12}$ allele caused a stronger effect: plasmid YEp(rsr1^{val12}) could suppress the growth defects not only of the *cdc25*(Ts) mutation (Fig. 4C, row 3) but also of the *cdc25* deletion mutation (data not shown) and of the *RAS2*(Ts) mutation (Fig. 4C, row 2). However, YEp(rsr1^{val12}) could not suppress the adenylyl cyclase-defective *cyr1*(Ts) mutation (Fig. 4C, row 1), suggesting that the effect of Rsr1p on the Ras pathway is at or upstream of the activation of this enzyme.

One possible explanation for the suppression of Ras pathway mutations by *RSR1* and *rsr1*$^{Val-12}$ is that Rsr1p may bind to and sequester the Ira proteins, thus allowing the Ras proteins to accumulate in their active GTP-bound state and bypassing the requirement for Cdc25p (Fig. 5A). In accord with this model, we found that the growth defect of the *RAS2*(Ts) mutant, like that of a *cdc25* mutant (56), could be suppressed by disruption of both *IRA* genes (Table 3). Another possibility is that Rsr1p may directly activate adenylyl cyclase in the place of the Ras proteins (Fig. 5B). In
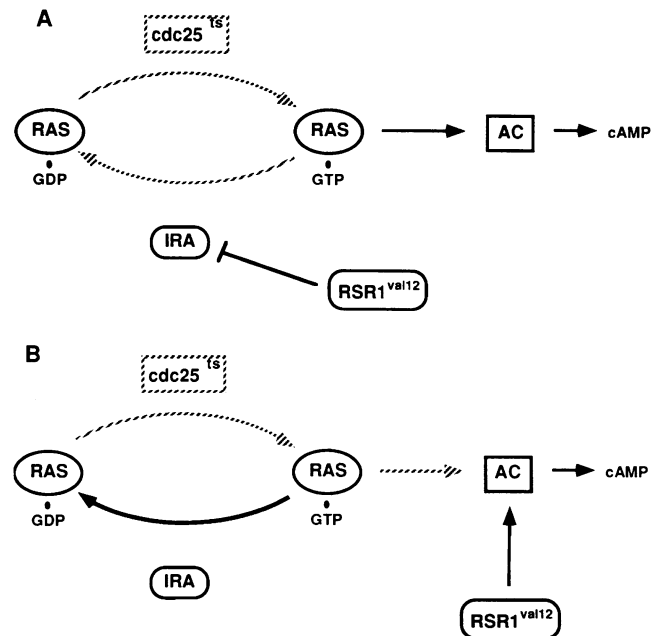
accord with this model, the growth defect of *ras1 RAS2*(Ts) cells was also suppressed by the *rsr1*$^{Val-12}$ plasmid (Table 3).

To investigate the second model further, we tested whether *rsr1*$^{Val-12}$ could suppress the lethality caused by a complete lack of both *RAS* genes; such suppression would be expected only under the second model (Fig. 5). A *ras1* strain (KMY45-2A) was crossed to a *ras2* strain carrying *rsr1*$^{Val-12}$ on a high-copy-number plasmid (KMY46-4Bp), and the haploid progeny obtained by tetrad analysis were analyzed for the genetic markers corresponding to the *ras* mutations and to the plasmid. Table 4 shows that the segregants predicted by meiotic recombination were obtained and that, as expected, spores carrying both *ras* mutations were mostly nonviable. The only viable *ras1 ras2* segregants observed carried the *rsr1*$^{Val-12}$ plasmid. To confirm that growth was indeed dependent on the *rsr1*$^{Val-12}$ plasmid, we transformed these segregants either with the *TRP1*-containing control plasmid YCpN1 or with the *TRP1*-containing plasmid YCp*RAS2*^{val19} and tested the ability of

TABLE 3. Suppression of *RAS2*(Ts) by *ira* mutations or by *rsr1*$^{Val-12}$ overexpression[a]

Strain	Relevant genotype	Growth at 37°C
RRY8-5B	*ira1 RAS2*(Ts)	−
RRY8-14B	*ira2 RAS2*(Ts)	−
RRY8-4A	*ira1 ira2 RAS2*(Ts)	+
RRY5-29B	*ras1 RAS2*(Ts) [YEp(rsr1^{Val12})]	+

[a] The strains used are described in Table 1. Suppression of the *RAS2*(Ts) temperature-sensitive growth phenotype was tested by streaking cells on both rich and selective media and incubating them for 3 days at 37°C.

TABLE 4. Rescue of *ras1 ras2* mutants by YEp(rsr1^{val12})^a

Genotype	No. of spores	
	Viable	Nonviable
RAS1 RAS2	8	0
ras1 RAS2	22	0
RAS1 ras2	22	0
ras1 ras2	0	5
ras1 ras2 [YEp(rsr1^{val12}]	3	0

^{a} Strains KMY45-2A and KMY46-4Bp (Table 1) were crossed, and 15 tetrads containing three or four viable spores were analyzed. The genotypes of viable spores were assigned by scoring the selectable markers associated with the gene disruptions or with the plasmid; the genotypes of nonviable spores with inferred from the genotypes of their sister segregants. The presence or absence of plasmid is indicated only for the *ras1 ras2* segregants.

TABLE 5. Dependence of *ras1 ras2* mutants on either *rsr1*$^{Val-12}$ or *RAS2*$^{Val-19}$ for viability^a

Strain	Additional plasmid	Proportion of colonies that were:	
		Leu$^-$	Trp$^-$
RRY9-2D	YCpN1	0/300	60/300
	YCp*RAS2*^{val19}	76/370	54/370
RRY9-26A	YCpN1	0/400	80/400
	YCp*RAS2*^{val19}	146/277	13/277

^{a} Two *ras1 ras2* [YEp(rsr1^{val12})] segregants from the cross of KMY45-2A to KMY46-4Bp (Tables 1 and 4) were transformed with the additional plasmids indicated (Table 2), grown for several generations in YPD medium at 25°C, and tested by replica plating for the loss of YEp(rsr1^{val12}) (Leu$^-$ colonies) or of the additional plasmid (Trp$^-$ colonies).

the transformants to segregate the plasmids upon growth in rich medium. With the transformants carrying the control plasmid in addition to the *rsr1*$^{Val-12}$ plasmid, we found no Leu$^-$ segregants although Trp$^-$ segregants were obtained in the expected frequency (Table 5). In contrast, when the *RAS2*$^{Val-19}$ plasmid was present in addition to the *rsr1*$^{Val-12}$ plasmid, Leu$^-$ as well as Trp$^-$ segregants were observed (Table 5). However, no Leu$^-$ Trp$^-$ clones were obtained. Thus, the cells could segregate either the *rsr1*$^{Val-12}$ plasmid or the *RAS2*$^{Val-19}$ plasmid, but not both, indicating that the viability of *ras1 ras2* cells was indeed supported by the *rsr1*$^{Val-12}$ plasmid.

A similar experiment showed that wild-type *RSR1* did not suffice for suppression of the *ras1 ras2* double mutation. When RRY9-2D in which YCp*RAS2*^{val19} had been substituted for YEp(rsr1^{val12}) (see above and Table 5) was transformed with YEp(RSR1) and grown on rich medium, Leu$^-$ segregants but no Trp$^-$ segregants were observed (data not shown).

Stimulation of adenylyl cyclase by *rsr1*$^{Val-12}$. To confirm that *rsr1*$^{Val-12}$ has a direct effect on the adenylyl cyclase complex, we tested the ability of the *ras1 ras2* cells carrying the *rsr1*$^{Val-12}$ plasmid to produce cAMP. In two steps of plasmid swapping like that of Table 5, the *rsr1*$^{Val-12}$ plasmid of strain RRY9-2D was replaced first by YCp*RAS2*^{val19} and then by YEp*TPK1*, which carries gene *TPK1* (encoding one isoform of the catalytic subunit of cAMP-dependent protein kinase). Overexpression of *TPK1* is known to support growth of cells lacking both *RAS* genes by activating events downstream of the cAMP formation step (13, 59). As reported previously (56), cAMP was not detectable in such downstream-activated cells (Fig. 6B). In contrast, *rsr1*$^{Val-12}$ conferred on these cells the ability to produce cAMP to levels about half those of wild-type cells (Fig. 6). Thus, *rsr1*$^{Val-12}$ can support the growth of *ras1 ras2* cells by directly stimulating the adenylyl cyclase complex and thereby bypassing the normal requirement for the Ras proteins.

DISCUSSION

Roles of Rsr1p and related proteins in cellular morphogenesis. Analysis of the *rsr1*$^{Val-12}$ and *rsr1*$^{Asn-16}$ mutants has provided some significant insights into the normal roles of Rsr1p and functionally related proteins. First, the observation that a wild-type strain harboring a low-copy-number *rsr1*$^{Asn-16}$ plasmid shows the same phenotype (random budding pattern) as an *rsr1* deletion strain indicates that the *rsr1*$^{Asn-16}$ allele encodes a dominant interfering protein, as

expected. More important, the viability of this strain, and even of a strain harboring *rsr1*$^{Asn-16}$ on a high-copy-number plasmid, suggests that the nonlethality of the *rsr1* deletion cannot be explained simply by functional redundancy of *RSR1*; if this were the case, the dominant interfering protein would presumably be lethal. In other words, *RSR1* appears to function only in bud site selection, not in the subsequent essential steps of polarity establishment and bud formation. This role contrasts with those of *CDC24* and *CDC42*. Mutation (*CDC24*) or overexpression (*CDC42*) of these genes can also randomize bud position (27, 53), but a loss of either gene product leads to a lethal inability to establish cell polarity (1, 23, 27, 53). One attractive model is that Rsr1p is one of a set of proteins (14, 15) that communicates the positional information for normal bud site selection to a second set of proteins that includes Cdc24p and Cdc42p; these latter proteins would then direct the formation of the polarized cytoskeletal assemblies necessary for bud formation. In the absence of the normal positional information, the Cdc24p/Cdc42p set of proteins would simply direct cytoskeletal assembly to a random site on the cell surface.

Second, the observation that an *rsr1*$^{Val-12}$ plasmid does not correct the random budding pattern of an *rsr1* deletion strain suggests that the normal function of Rsr1p depends on a cycling between GTP-bound and GDP-bound states and not simply on having a sufficient fraction of the protein in an active (GTP-bound) conformation. The observation that *rsr1*$^{Val-12}$ plasmids can interfere with bud site selection even in a wild-type strain further supports this view. In this respect, Rsr1p appears to resemble GTP-binding proteins involved in vectorial processes (8, 9), such as Sec4p and Yptlp, more than it does Ras proteins. One plausible model is that the switch from the GDP-bound to the GTP-bound form might signal the successful completion of one stage (and/or readiness for the next stage) in the assembly of a prebudding protein complex at the prospective budding site.

Finally, the observation that a high-copy-number *rsr1*$^{Val-12}$ plasmid suppresses the lethal *cdc24* mutation sheds some light on the relationship between Rsr1p and Cdc24p. As Rsr1p^{Val-12} appears not to have normal Rsr1p function (see above), it does not appear that this suppression can be explained by the hypothesis that a constitutive activation of Rsr1p bypasses the need for Cdc24p function (as might be the case if Rsr1p functioned downstream of, or was activated by, Cdc24p). This conclusion is supported by the observation that high-copy-number *rsr1*$^{Val-12}$ actually suppresses less well than does high-copy-number *RSR1* (data not shown). A plausible alternative hypothesis is that the suppression of *cdc24* results from the increased amount of Rsr1p
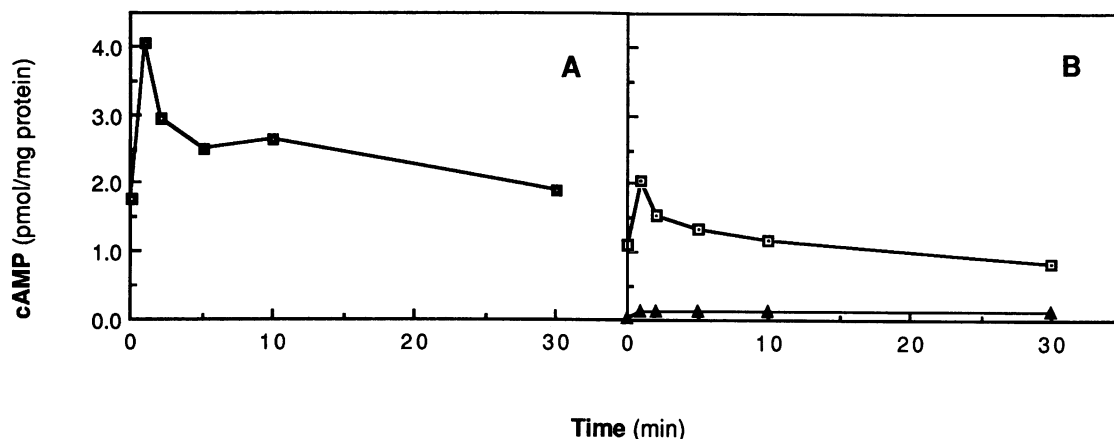
Time (min)

FIG. 6. Restoration of cAMP levels in *ras1 ras2* double mutants by *rsr1*$^{Val-12}$. Cells of strain RRY9-2D (Table 1) were grown to stationary phase in YPD liquid medium at 25°C. One aliquot of this culture was then plated on YPD medium; when colonies appeared, they were replica plated onto selective media to confirm the dependency of growth on the *rsr1*$^{Val-12}$ plasmid. A second aliquot of the same culture was washed twice with water and then incubated for 2 h at 25°C in a buffer containing 10 mM morpholine ethanesulfonic acid (pH 6.5) and 0.1 mM EDTA. Glucose was then added to a final concentration of 25 mM, and after various periods of time 0.5-ml aliquots were transferred to 0.5 ml of 10% (wt/vol) trichloroacetic acid. The cAMP contents were then measured as described in Materials and Methods. The data shown (B; □) are from a single analysis but are representative of results obtained in repeated experiments with RRY9-2D as well as with strain RRY9-26A (Table 1). Similar experiments were performed with the congenic wild-type strain RRY9-19B (Table 1) (A) and with a derivative of RRY9-2D in which the *rsr1*$^{Val-12}$ plasmid had been replaced by the *TPK1*-containing plasmid YEp*TPK1* (see text and Table 2) (B; ▲).

(either wild type or the Val12 form) stabilizing an enfeebled Cdc24p by direct protein-protein interaction. This hypothesis is consistent with the observation that suppression of *cdc24* by either *RSR1* or *rsr1*$^{Val-12}$ is dosage dependent: low-copy-number *RSR1* plasmids suppress less well than do high-copy-number *RSR1* plasmids (4a, 5), and low-copy-number *rsr1*$^{Val-12}$ plasmids suppress little or not at all (see Results). Such an interaction between Rsr1p and Cdc24p could, of course, be a component of the mechanism for communicating positional information, as suggested above. Perhaps it is the complex of Cdc24p and the GTP-bound form of Rsr1p (as presumably represented by Rsr1p^{Val-12}) that normally participates in the next stage in the assembly of the prebudding complex; with wild-type Rsr1p, the Cdc24p-Rsr1p[GTP] complex would form only at the proper site. In this regard, it is also of interest that *rsr1*$^{Asn-16}$ cannot suppress the *cdc24* mutation. It seems likely that Rsr1p^{Asn-16} is unable to interact with Cdc24p (thus explaining its inability to suppress) but can still compete with Rsr1p for binding to other elements of the position-signalling apparatus (thus explaining its dominant effect on budding pattern). The ability of *rsr1*$^{Asn-16}$ to interfere with the suppression of *cdc24* by *RSR1* could be explained if this competition prevents Rsr1p from being switched to the GTP-bound form and if only Rsr1p[GTP] is competent to interact with Cdc24p. The observation that *rsr1*$^{Asn-16}$ cannot interfere with the suppression of *cdc24* by *rsr1*$^{Val-12}$ is consistent with this interpretation.

Functional homology between Rsr1p and Krev-1 p21. Two lines of evidence suggest that the structural similarity between Rsr1p and Krev-1 p21 is reflected in a functional homology. First, expression of Krev-1 in yeast cells can randomize the budding pattern of wild-type cells, presumably by interacting with one or more normal components of the machinery for bud site selection and thus interfering with this process. Second, expression of Krev-1 p21 can suppress a *cdc24* mutation. Although overexpression of the GTP-binding protein Cdc42p can also both randomize bud position (27) and suppress a *cdc24* mutation (5), such effects are

not generic consequences of overexpressing Ras-like proteins in yeast cells, as no similar effects were observed with plasmids containing a variety of other *ras*-like genes (see Results).

The two effects noted for Krev-1 expression in yeast cells might actually have a common basis; that is, binding of Krev-1 p21 to Cdc24p (stabilizing the mutant Cdc24p and potentiating subsequent steps in the assembly of the prebudding complex) might occur without reference to the positional signals that would normally be necessary (perhaps by promoting the exchange of GTP for GDP) for the binding of Rsr1p to Cdc24p. Such an effect would be enhanced if Krev-1 p21 were not subject to control by the (putative) Rsr1p GAP. This would cause the mammalian protein to accumulate in its GTP-bound state and thus behave more like Rsr1p^{Val-12} than like wild-type Rsr1p. Such a lack of control would be similar to the inability of yeast Ras GAP (the products of the *IRA* genes) to affect the intrinsic GTPase activity of mammalian Ha-*ras* p21 (54, 57). Regardless of the detailed interpretation, the observations cited do appear to indicate a functional homology between Rsr1p and Krev-1 p21.

Mechanism of Krev-1 reversal of *ras*-induced transformation. Given the apparent functional homology between Rsr1p and Krev-1 p21, we examined whether Rsr1p could interact with the Ras pathway in *S. cerevisiae*. Although no suppression of the activated *RAS2*$^{Val-19}$ allele was observed, overexpression of *rsr1*$^{Val-12}$ could suppress various mutations that inactivate the Ras pathway. The observation that *rsr1*$^{Val-12}$ was more effective in this suppression than was *RSR1* parallels the observation that in mammalian cells, the tumor suppression activity of Krev-1 is potentiated by substitution of Val for Gly at position 12 (32), a modification analogous to that in Rsr1p^{Val-12}. The *cyr1*(Ts) defect was not suppressed by *rsr1*$^{Val-12}$, indicating that the suppression involved a direct effect on the Ras pathway rather than an activation of an independent growth-stimulating pathway. Indeed, the stimulation of cAMP formation in the absence of the Ras proteins showed that Rsr1p can directly activate the

adenylyl cyclase complex. In addition, the suppression of the *RAS2*(Ts) mutation by elimination of both Ira proteins raises the possibility that Rsr1p might also affect the Ras pathway by interacting with and blocking the action of the Ira proteins. Such an interaction would not be surprising given the exact conservation between Rsr1p and the Ras proteins of the "effector domain", which is believed to be the site of interaction between *ras* p21 and *ras* GAP in mammalian cells (2, 12). However, as yet we have no experimental evidence that bears on this possibility.

The ability of Rsr1p^{Val-12} to stimulate adenylyl cyclase, the Ras effector in *S. cerevisiae*, suggests that K*rev*-1 may suppress *ras*-induced transformation in mammalian cells by competing with *ras* p21 for an effector. This effector might or might not be *ras* GAP (22, 39, 61), which has been reported to bind tightly to K*rev*-1 p21 in vitro (18, 24). It is interesting that K*rev*-1 can suppress the effects of *ras* activation in mammalian cells, whereas in yeast cells it is a loss of Ras pathway function that is suppressed by *RSR1* (or *rsr1*$^{Val-12}$). This difference appears similar to that observed in a recent study of the expression of K*rev*-1 in *S. cerevisiae* and *Schizosaccharomyces pombe* (62). In *S. cerevisiae*, K*rev*-1 was observed to interfere with *RAS2* function, whereas in *S. pombe*, K*rev*-1 p21 could stimulate some of the Ras effectors. Taken together, these results suggest that a variety of cross-pathway effects involving closely related members of the Ras family are possible. However, the precise direction of these effects is difficult to predict in individual cases. In particular, the interaction with Ras effectors may sometimes be stimulatory and sometimes inhibitory. Presumably, detailed comparative studies of the effector-binding domains of the various proteins will eventually rationalize these differences.

REFERENCES

1. Adams, A. E. M., D. I. Johnson, R. M. Longnecker, B. F. Sloat, and J. R. Pringle. 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. 111:131–142.
2. Adari, H., D. R. Lowy, B. M. Willumsen, C. J. Der, and F. McCormick. 1988. Guanosine triphosphatase activating protein (GAP) interacts with the p21 *ras* effector binding domain. Science 240:518–521.
3. Ballester, R., T. Michaeli, K. Ferguson, H-P. Xu, F. McCormick, and M. Wigler. 1989. Genetic analysis of mammalian GAP expressed in yeast. Cell 59:681–686.
4. Barbacid, M. 1987. *ras* genes. Annu. Rev. Biochem. 56:779–828.
4a. Bender, A. Unpublished data.
5. Bender, A., and J. R. Pringle. 1989. Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the *ras*-related gene *RSR1*. Proc. Natl. Acad. Sci. USA 86:9976–9980.
6. Bender, A., and G. F. Sprague, Jr. 1989. Pheromones and pheromone receptors are the primary determinants of mating specificity in the yeast *Saccharomyces cerevisiae*. Genetics 121:463–476.
7. Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis. 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene 8:17–24.
8. Bourne, H. R. 1988. Do GTPases direct membrane traffic in secretion? Cell 53:669–671.
9. Bourne, H. R., D. A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cellular functions. Nature (London) 348:125–132.
10. Broach, J. R., J. N. Strathern, and J. B. Hicks. 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene 8:121–133.
11. Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler. 1987. The *S. cerevisiae CDC25* gene product regulates the *RAS*/adenylate cyclase pathway. Cell 48:789–799.
12. Calés, C., J. F. Hancock, C. J. Marshall, and A. Hall. 1988. The cytoplasmic protein GAP is implicated as the target for regulation by the *ras* gene product. Nature (London) 332:548–551.
13. Cannon, J. F., and K. Tatchell. 1987. Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. 7:2653–2663.
14. Chant, J., K. Corrado, J. R. Pringle, and I. Herskowitz. 1991. *BUD5*, encoding a putative GTP/GDP exchange factor, is necessary for bud-site selection and interacts with bud formation gene *BEM1*. Cell 65:1213–1224.
15. Chant, J., and I. Herskowitz. 1991. Genetic control of bud-site selection in yeast by a set of gene products that comprise a morphogenetic pathway. Cell 65:1203–1212.
16. Crechet, J.-B., P. Poullet, M.-Y. Mistou, A. Parmeggiani, J. Camonis, E. Boy-Marcotte, F. Damak, and M. Jacquet. 1990. Enhancement of the GDP-GTP exchange of RAS proteins by the carboxyl-terminal domain of SCD25. Science 248:866–868.
17. DeFeo-Jones, D., K. Tatchell, L. C. Robinson, I. S. Sigal, W. C. Vass, D. R. Lowy, and E. M. Scolnick. 1985. Mammalian and yeast *ras* gene products: biological function in their heterologous systems. Science 228:179–184.
18. Frech, M., J. John, V. Pizon, P. Chardin, A. Tavitian, R. Clark, F. McCormick, and A. Wittinghofer. 1990. Inhibition of GTPase activating protein stimulation of Ras-p21 GTPase by the K*rev*-1 gene product. Science 249:169–171.
19. Gibbs, J. B., and M. S. Marshall. 1989. The *ras* oncogene—an important regulatory element in lower eucaryotic organisms. Microbiol. Rev. 53:171–185.
20. Gibbs, J. B., M. D. Schaber, M. S. Marshall, E. M. Scolnick, and I. S. Sigal. 1987. Identification of guanine nucleotides bound to *ras*-encoded proteins in growing yeast cells. J. Biol. Chem. 262:10426–10429.
21. Hall, A. 1990. The cellular functions of small GTP-binding proteins. Science 249:635–640.
22. Hall, A. 1990. ras and GAP—who's controlling whom? Cell 61:921–923.
23. Hartwell, L. H., R. K. Mortimer, J. Culotti, and M. Culotti. 1973. Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. Genetics 74:267–286.
24. Hata, Y., A. Kikuchi, T. Sasaki, M. D. Schaber, J. B. Gibbs, and Y. Takai. 1990. Inhibition of the *ras* p21 GTPase-activating protein-stimulated GTPase activity of c-Ha-*ras* p21 by *smg* p21 having the same putative effector domain as *ras* p21s. J. Biol. Chem. 265:7104–7107.
25. Holland, J. P., and M. J. Holland. 1979. The primary structure of a glyceraldehyde-3-phosphate dehydrogenase gene from *Saccharomyces cerevisiae*. J. Biol. Chem. 254:9839–9845.
26. Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. 153:163–168.
27. Johnson, D. I., and J. R. Pringle. 1990. Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. J. Cell Biol. 111:143–152.

28. Jones, S., M.-L. Vignais, and J. R. Broach. 1991. The *CDC25* protein of *Saccharomyces cerevisiae* promotes exchange of guanine nucleotides bound to Ras. Mol. Cell. Biol. 11:2641–2646.

29. Kataoka, T., D. Broek, and M. Wigler. 1985. DNA sequence and characterization of the *S. cerevisiae* gene encoding adenylate cyclase. Cell 43:493–505.

30. Kataoka, T., S. Powers, C. McGill, O. Fasano, J. Strathern, J. Broach, and M. Wigler. 1984. Genetic analysis of yeast *RAS1* and *RAS2* genes. Cell 37:437–445.

31. Kawata, M., Y. Matsui, J. Kondo, T. Hishida, Y. Teranishi, and Y. Takai. 1988. A novel small molecular weight GTP-binding protein with the same putative effector domain as the *ras* proteins in bovine brain membranes. J. Biol. Chem. 263:18965–18971.

32. Kitayama, H., T. Matsuzaki, Y. Ikawa, and M. Noda. 1990. Genetic analysis of the Kirsten-*ras*-revertant 1 gene: potentiation of its tumor suppressor activity by specific point mutations. Proc. Natl. Acad. Sci. USA 87:4284–4288.

33. Kitayama, H., Y. Sugimoto, T. Matsuzaki, Y. Ikawa, and M. Noda. 1989. A *ras*-related gene with transformation suppressor activity. Cell 56:77–84.

34. Levitzki, A. 1990. GTP-GDP exchange proteins. Science 248:794.

35. Lillie, S. H., and J. R. Pringle. 1980. Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. J. Bacteriol. 143:1384–1394.

36. Madaule, P., R. Axel, and A. M. Myers. 1987. Characterization of two members of the *rho* family from the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 84:779–783.

37. Marshall, M. S., J. B. Gibbs, E. M. Scolnick, and I. S. Sigal. 1987. Regulatory function of the *Saccharomyces cerevisiae RAS* C terminus. Mol. Cell. Biol. 7:2309–2315.

38. McAlister, L., and M. J. Holland. 1985. Isolation and characterization of yeast strains carrying mutations in the glyceraldehyde-3-phosphate dehydrogenase genes. J. Biol. Chem. 260:15013–15018.

39. McCormick, F. 1989. *ras* GTPase activating protein: signal transmitter and signal terminator. Cell 56:5–8.

40. Nakafuku, M., T. Obara, K. Kaibuchi, I. Miyajima, A. Miyajima, H. Ito, S. Nakamura, K.-I. Arai, K. Matsumoto, and Y. Kaziro. 1988. Isolation of a second yeast *Saccharomyces cerevisiae* gene (*GPA2*) coding for guanine nucleotide-binding regulatory protein: studies on its structure and possible functions. Proc. Natl. Acad. Sci. USA 85:1374–1378.

41. Nakayama, N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. 4:2643–2648.

42. Pizon, V., P. Chardin, I. Lerosey, B. Olofsson, and A. Tavitian. 1988. Human cDNAs rap1 and rap2 homologous to the *Drosophila* gene Dras3 encode proteins closely related to ras in the 'effector' region. Oncogene 3:201–204.

43. Powers, S., T. Kataoka, O. Fasano, M. Goldfarb, J. Strathern, J. Broach, and M. Wigler. 1984. Genes in *S. cerevisiae* encoding proteins with domains homologous to the mammalian *ras* proteins. Cell 36:607–612.

44. Powers, S., K. O'Neill, and M. Wigler. 1989. Dominant yeast and mammalian *RAS* mutants that interfere with *CDC25*-dependent activation of wild-type *RAS* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 9:390–395.

45. Pringle, J. R., R. A. Preston, A. E. M. Adams, T. Stearns, D. G. Drubin, B. K. Haarer, and E. W. Jones. 1989. Fluorescence microscopy methods for yeast. Methods Cell Biol. 31:357–435.

46. Robinson, L. C., J. B. Gibbs, M. S. Marshall, I. S. Sigal, and K. Tatchell. 1987. *CDC25*: a component of the RAS-adenylate cyclase pathway in *Saccharomyces cerevisiae*. Science 235:1218–1221.

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

48. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

49. Sanger, F., S. Nicklen, and A. R. Coulsen. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.

50. Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

51. Sigal, I. S., J. B. Gibbs, J. S. D'Alonzo, and E. M. Scolnick. 1986. Identification of effector residues and a neutralizing epitope of Ha-*ras*-encoded p21. Proc. Natl. Acad. Sci. USA 83:4725–4729.

52. Sigal, I. S., J. B. Gibbs, J. S. D'Alonzo, G. L. Temeles, B. S. Wolanski, S. H. Socher, and E. M. Scolnick. 1986. Mutant *ras*-encoded proteins with altered nucleotide binding exert dominant biological effects. Proc. Natl. Acad. Sci. USA 83:952–956.

53. Sloat, B. F., A. Adams, and J. R. Pringle. 1981. Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. 89:395–405.

54. Tanaka, K., B. K. Lin, D. R. Wood, and F. Tamanoi. 1991. IRA2, an upstream negative regulator of RAS in yeast, is a RAS GTPase-activating protein. Proc. Natl. Acad. Sci. USA 88:468–472.

55. Tanaka, K., K. Matsumoto, and A. Toh-e. 1988. Dual regulation of the expression of the polyubiquitin gene by cyclic AMP and heat shock in yeast. EMBO J. 7:495–502.

56. Tanaka, K., K. Matsumoto, and A. Toh-e. 1989. *IRA1*, an inhibitory regulator of the *RAS*-cyclic AMP pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 9:757–768.

57. Tanaka, K., M. Nakafuku, T. Satoh, M. S. Marshall, J. B. Gibbs, K. Matsumoto, Y. Kaziro, and A. Toh-e. 1990. *S. cerevisiae* genes *IRA1* and *IRA2* encode proteins that may be functionally equivalent to mammalian *ras* GTPase-activating protein. Cell 60:803–807.

58. Tanaka, K., M. Nakafuku, F. Tamanoi, Y. Kaziro, K. Matsumoto, and A. Tohe. 1990. *IRA2*, a second gene of *Saccharomyces cerevisiae* that encodes a protein with a domain homologous to mammalian *ras* GTPase-activating protein. Mol. Cell. Biol. 10:4303–4313.

59. Toda, T., S. Cameron, P. Sass, M. Zoller, and M. Wigler. 1987. Three different genes in *S. cerevisiae* encode the catalytic subunit of the cAMP-dependent protein kinase. Cell 50:277–287.

60. Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Kataoka, D. Broek, S. Cameron, J. Broach, K. Matsumoto, and M. Wigler. 1985. In yeast, *RAS* proteins are controlling elements of adenylate cyclase. Cell 40:27–36.

61. Wigler, M. H. 1990. GAPs in understanding Ras. Nature (London) 346:696–697.

62. Xu, H-P., Y. Wang, M. Riggs, L. Rodgers, and M. Wigler. 1990. Biological activity of the mammalian RAP genes in yeast. Cell Regul. 1:763–769.

63. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.