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Dominant Yeast and Mammalian *RAS* Mutants That Interfere with the *CDC25*-Dependent Activation of Wild-Type *RAS* in *Saccharomyces cerevisiae*

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Two mutant alleles of *RAS2* were discovered that dominantly interfere with wild-type *RAS* function in the yeast *Saccharomyces cerevisiae*. An amino acid substitution which caused the dominant interference was an alanine for glycine at position 22 or a proline for alanine at position 25. Analogous mutations in human H-*ras* also dominantly inhibited *RAS* function when expressed in yeast cells. The inhibitory effects of the mutant *RAS2* or H-*ras* genes could be overcome by overexpression of *CDC25*, but only in the presence of wild-type *RAS*. These results suggest that these mutant *RAS* genes interfere with the normal interaction of RAS and CDC25 proteins and suggest that this interaction is direct and has evolutionarily conserved features.

RAS proteins share a feature common to other guanine-nucleotide-binding proteins in that the GTP-bound form of the protein, but not the GDP-bound form, activates the target effector (1, 20). Biochemical evidence in support of this comes from studies in *Saccharomyces cerevisiae* in which RAS2 protein has been shown to activate adenylate cyclase when bound to GTP, but not when bound to GDP (11, 15, 16), and from studies of frog oocytes, in which only the GTP-bound form of RAS protein induces oocyte maturation (44). It is therefore of interest to identify components that can control the guanine-nucleotide bound to RAS proteins and thereby control its activity. Recently, a protein has been identified which accelerates hydrolysis of GTP by mammalian RAS proteins (19, 44). In other guanine-nucleotide-binding protein systems, the conversion of GDP-bound to GTP-bound protein is controlled by the activity of distinct nucleotide exchange catalysts (20, 41). There is some preliminary evidence that, in *S. cerevisiae*, the CDC25 protein may fulfill this role for RAS1 and RAS2 proteins (6, 10, 38).

The *CDC25* gene was originally identified by a temperature-sensitive (Ts) mutation that causes *S. cerevisiae* to arrest their progression through the cell cycle uniformly in G1 (37). This terminal arrest phenotype is exhibited by other cell cycle mutations such as *cdc35*, which is allelic to *CYR1*, the gene that encodes adenylate cyclase (3, 8, 24, 33). *CDC25* mutations significantly reduce cyclic AMP levels (6, 7, 32). Epistasis studies have suggested that the *CDC25* gene product acts upstream of RAS proteins in the control of adenylate cyclase (6, 31, 38). Based on the GTP requirement for adenylate cyclase activity in membranes prepared from *cdc25* mutants, yeast RAS proteins appear to require *CDC25* function to become bound to GTP (6).

In this report, we have utilized dominant-interfering mutants of *RAS2* and H-*ras* to further explore the relationship between CDC25 and RAS proteins.

MATERIALS AND METHODS

Strains. Wild-type strains SP1 (*MATα leu2 ura3 his3 trp1 ade8 gal2 can1*) and RS16-4C (*MATα ura3 his3 trp1 ade2 ade8*) were used in these studies (36). The *ras1::URA3* $RAS2^{Val-19}$ strain PT1-6 was derived from SP1 by transformation (36). PT1-6 is the parental strain for the STS revertant series, including STS13, STS35, and STS37. The *ras2::URA3* strain KP-2 was derived from SP1 by transformation (26). RS60-3A (*MATα* $RAS2^{Val-19, Ala-22}$ *ura3 his3 ade8 trp1*) was derived from a cross between STS13 and RS16-4C. SDR-3 is a His^+ transformant of SP1 containing plasmid p*GAL10*-H-$ras^{Val-12, Ala-15}$ integrated at the *HIS3* locus (see below).

Cloning and assaying the activity of the dominant *RAS2*(Ts) alleles. Genomic DNA from strains STS13, STS35, and STS37 was digested to completion with *Eco*RI and *Hin*dIII, and DNA libraries were constructed in pUC9 from size-selected DNA. The *RAS2* locus is contained within a 3.0-kilobase-pair *Eco*RI-*Hin*dIII fragment (35). The libraries were screened with a *RAS2* probe. Positive candidates pSTS13, pSTS35, and pSTS37 were isolated from their respective libraries.

Biological activity of the cloned genes was assayed in the following manner. The 3.0-kilobase-pair *Eco*RI-*Hin*dIII fragments were cloned into the *URA3* integration vector YIp5 (2), and the resultant plasmids were digested with *Cla*I, which cleaves within the *RAS2* locus (35). The linearized plasmids were used to transform strain SP1, and the Ura^+ transformants were tested for growth at 37°C. A YIp5-based plasmid containing wild-type *RAS2* served as a negative control.

Localization of the *RAS2*(Ts) activity. *Pst*I cleaves the *RAS2* coding sequence within codons 65 and 66 (35). Chimeric plasmids encoding the first 65 amino acids from the *RAS2*(Ts) alleles and the remaining 257 amino acids from $RAS2^{Val-19}$ were constructed by transfer of the appropriate *Eco*RI-*Pst*I fragments of the pSTS plasmids into vector arms provided by p$RAS2^{Val-19}$. Likewise, chimeric plasmids encoding the C-terminal 257 amino acids of the *RAS2*(Ts) alleles and the first 65 amino acids of $RAS2^{Val-19}$ were constructed by transfer of the appropriate *Pst*I-*Hin*dIII fragments of the pSTS plasmids into vector arms provided by p$RAS2^{Val-19}$. Biological activity was assayed as described above.

Oligonucleotide-directed mutagenesis. The *Eco*RI-*Pst*I fragments of p*RAS2* and p$RAS2^{Val-19}$ (26) were transferred

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into M13 vectors. Single-stranded DNA was annealed to oligonucleotides, extended, and transformed into *ung Escherichia coli* as described before (27, 45). The candidate mutants were then sequenced. Plasmids p*RAS2*$^{Ala-22}$, p*RAS2*$^{Pro-25}$, p*RAS2*$^{Val-19, Ala-22}$, and p*RAS2*$^{Val-19, Pro-25}$ were constructed by transfer of the *Eco*RI-*Pst*I fragments from the mutant pUC plasmids into vector arms provided by p*RAS2*. Biological activity was assayed as described above.

The oligonucleotide-derived mutants of H-*ras* were constructed in pUC vectors with *Sal*I-*Bam*HI fragments containing either H-*ras*$^{Val-12}$ or wild-type H-*ras*, as described in reference 25. The mutant H-*ras* alleles were transferred into the *GAL10* expression vector YEP51 (4) as described previously (25). The *HIS3*$^+$ integration vectors p*GAL10*-H-*ras*$^{Ala-15}$ and p*GAL1*-H-*ras*$^{Val-12, Ala-15}$ were constructed by transferring the *Eco*RI-*Xba*I fragments containing the mutant *GAL10*-H-*ras* alleles into a pBR322-based vector containing *HIS3*.

Other plasmids. YEp*RAS2*-1, YEp*RAS2*$^{Val-19}$, YEp*CDC 25*-1, and YEp*TPK1* are YEp13-based plasmids that have been described previously (6, 35, 43). pRG2 contains the 3.0-kilobase-pair *Eco*RI-*Hin*dIII fragment from p*RAS2* inserted into the intermediate copy number vector pHV1 (J. Nikawa, unpublished data). pHV1 contains the polylinker from pUC18, the *HIS3* gene, and an *Hpa*I-*Hin*dIII fragment from 2μm including the origin of replication but lacking *REP3* (5).

RESULTS

Isolation and characterization of temperature-sensitive *RAS2* alleles. We have isolated several phenotypic revertants of the *ras1*$^-$ *RAS2*$^{Val-19}$ strain PT1-6, using heat shock survival after stationary phase as a selective assay (36). As reported previously, 43 heat-shock-resistant revertants have been isolated at 25°C that have the additional property of temperature-sensitive growth at 35°C (36). Eighteen of these 43 temperature-sensitive revertants appeared to be intragenic revertants for the following reason: when mated to cells with the *RAS2*$^{Val-19}$ mutant allele, heat-shock-sensitive diploid cells were formed and when mated to wild-type cells, heat-shock-resistant diploid cells were formed. These results are consistent with the occurrence of suppressor lesions within the *RAS2*$^{Val-19}$ gene carried by PT1-6.

Three of these 18 revertants (STS1, STS3, and STS8) were mated to wild-type cells, and the resultant diploids were sporulated and dissected. Tetrad analysis confirmed the intragenic nature of the phenotypic reversion (4:0 segregation of heat shock resistance to heat shock sensitivity in 35 complete tetrads). This analysis also indicated that the temperature-sensitive alleles segregated independently of *RAS1* but required the absence of *RAS1* (*ras1*::*URA3*) for phenotypic penetrance (31 of 70 *ras1*$^-$ haploids were temperature sensitive, and 0 of 70 *RAS1*$^+$ haploids were temperature sensitive). Thus, these three strains appeared to contain recessive, temperature-sensitive *ras2* alleles [*ras2*(Ts)]. Complementation tests with a mating strain constructed from one of these crosses showed that 15 of 18 of these revertants behaved as if they also contained recessive *ras2*(Ts) alleles. However, 3 of the 18 revertants behaved as if they contained dominant *RAS2*(Ts) alleles since they formed temperature-sensitive diploids when mated to strains containing wild-type *RAS* genes.

To confirm that these three particular revertants (STS13, STS35, and STS37) contained dominant temperature-sensitive *RAS2* alleles, two of them (SDS13 and STS37) were mated to a wild-type strain and the resultant diploids were subjected to tetrad analysis. In 10 complete tetrads from each cross, the temperature-sensitive phenotype segregated 2:2 despite the presence of wild-type *RAS1*. A *RAS1*$^+$ temperature-sensitive strain from the STS13 cross (RS60-12D) was mated to the *ras2*::*URA3* strain KP-2 to study linkage of the temperature-sensitive phenotype to the *RAS2* locus. The segregation pattern of Ura$^+$ and Ts$^-$ indicated complete linkage of the temperature-sensitive phenotype to the *RAS2* locus (no recombinants in 23 complete tetrads). We therefore concluded that STS13 contains a dominant temperature-sensitive *RAS2* allele [*RAS2*(Ts)]. Proof that the other two strains (STS35 and STS37) contain dominant *RAS2*^{ts} alleles comes from cloning experiments described in a later section. Thus, there are two classes of intragenic mutations of *RAS2*$^{Val-19}$ that revert the *RAS2*$^{Val-19}$ phenotype and have temperature-sensitive function: the predominant class (15 of 18), composed of typical recessive loss-of-function temperature-sensitive alleles; and the other class (3 of 18), composed of dominant *RAS2*(Ts) alleles.

To explore the nature of the dominant interference with growth, the three revertants containing dominant *RAS2*(Ts) alleles were transformed with various high-copy plasmids which overexpress genes of the *RAS*/cyclic AMP pathway. As a control, three strains containing recessive *ras2*(Ts) alleles (and *ras1*::*URA3*) were transformed with the same plasmids. Growth at 35°C was restored in all six of these mutant strains by overexpression of the adenylate cyclase gene *CYR1* (24) or by overexpression of the cyclic AMP-dependent protein kinase gene *TPK1* (43; see Table 1). Thus, downstream activation of the cyclic AMP pathway can overcome the temperature-sensitive block in cell growth imposed by both recessive *ras1*$^-$ *ras2*(Ts) mutations and the dominant *RAS2*(Ts) mutations. As expected, overexpression of *RAS1* or *RAS2* restored growth at 35°C to the *ras1*$^-$ *ras2*(Ts) mutant (Table 1). The temperature-sensitive defect of two of the dominant *RAS2*(Ts) mutants, however, was not overcome by overexpression of wild-type *RAS1* or *RAS2*, although the temperature-sensitive defect of STS37 was suppressed (Table 1). Even vast overexpression of wild-type *RAS2*, using the *GAL10* promoter, cannot reverse the temperature-sensitive defect of dominant *RAS2*(Ts) mutants STS13 and STS35 (data not shown). Some of the dominant *RAS2*(Ts) alleles are therefore capable of blocking *RAS* function even in the presence of a vast excess of wild-type *RAS*. Thus, it is unlikely that these dominant RAS2(Ts)

TABLE 1. Ability of overexpressed genes to suppress the temperature-sensitive defect of mutant *RAS2* strains

Strain[a]	Suppression of temperature-sensitive phenotype by high-copy plasmids[b]				
	RAS1	*RAS2*	*RAS2*$^{Val-19}$	*CYR1*	*TPK1*
STS1	+	+	+	+	+
STS3	+	+	+	+	+
STS8	+	+	+	+	+
STS13	−	−	+	+	+
STS35	−	−	+	+	+
STS37	+	+	+	+	+

[a] All STS strains were derived from PT1-6 (*ras1*$^-$ *RAS2*$^{Val-19}$) and contained temperature-sensitive *ras2* or *RAS2* alleles as described in the text.

[b] STS strains were transformed with YEp13-based vectors containing *RAS1*, *RAS2*, *RAS2*$^{Val-19}$, *CYR1*, or *TPK1* (see Materials and Methods). Five transformants from each group were patched onto SC-Leu plates and replica plated onto YPD plates for assessing growth at 35°C. YEp13 transformants served as a negative control.

proteins are interfering with wild-type RAS proteins by forming ineffective multimeric RAS protein complexes, a common mechanism of inhibition found in dominant-interfering mutants (22). The effect of the RAS2(Ts) protein from STS37, however, is competitively reversed by overexpression of wild-type *RAS*.

Overexpression of *RAS2*$^{Val-19}$ does overcome the temperature-sensitive block induced by all of the *RAS2*(Ts) alleles (Table 1). Furthermore, the heat-shock-sensitive phenotype induced by *RAS2*$^{Val-19}$ is not influenced by the presence of *RAS2*(Ts) alleles (data not shown). Thus, the dominant *RAS2*(Ts) alleles, although they are capable of blocking the function of wild-type *RAS* and, in some cases, overexpressed wild-type *RAS*, do not interfere with the function of activated *RAS2*$^{Val-19}$. In fact, even single-copy *RAS2*$^{Val-19}$ is epistatic to all of the dominant *RAS2*(Ts) alleles, since diploids formed between *RAS2*(Ts) strains and *RAS2*$^{Val-19}$ strains are fully heat shock sensitive and are not temperature sensitive for growth. It seems likely, therefore, that the dominant RAS2(Ts) proteins are blocking a step required for the activation of wild-type yeast RAS proteins and that they are not blocking a step involved in the interaction of RAS proteins with their target effector.

Molecular analysis of the dominant temperature-sensitive *RAS2* alleles. We cloned the *RAS2*(Ts) alleles from STS13, STS35, and STS37 by screening genomic libraries with a *RAS2* probe (see Materials and Methods). The cloned *RAS2*(Ts) alleles were transferred to the yeast integration vector YIp5 and transformed into yeast strain SP1 (see Materials and Methods). All three cloned *RAS2*(Ts) alleles were capable of dominantly inducing temperature-sensitive growth at 37°C (data not shown).

To localize the *RAS2* mutations that altered their properties, we made chimeras between the dominant *RAS2*(Ts) and wild-type *RAS2* genes (see Materials and Methods). The temperature-sensitive lesions for all three alleles were localized to within DNA encoding the first 66 N-terminal amino acids. We sequenced the three cloned genes within this region, and in each case a single nucleotide change, resulting in a single amino acid substitution, was detected. In STS13 and STS35, a G-to-C transversion in the 22nd codon (GGT to GCT) resulted in a substitution of alanine for glycine. In STS37, a G-to-C transversion in the 25th codon (GCT to CCT) resulted in a substitution of proline for alanine. To confirm that these single amino acid substitutions caused the dominant temperature-sensitive phenotype, and to test whether these substitutions would cause a dominant temperature-sensitive phenotype if position 19 encoded glycine (wild type) instead of valine, we constructed four *RAS2* genes by oligonucleotide-directed mutagenesis: *RAS2*$^{Val-19, Ala-22}$, *RAS2*$^{Val-19, Pro-25}$, *RAS2*$^{Ala-22}$, and *RAS2*$^{Pro-25}$ (see Materials and Methods). All four of these genes were transferred into the yeast integration vector YIp5 and transformed into strain SP1, and all four genes induced temperature-sensitive growth (data not shown). We conclude, therefore, that the amino acid substitution observed in the *RAS2*(Ts) alleles of STS13 and STS35 (a glycine-to-alanine substitution at position 22) and the amino acid substitution observed in the *RAS2*(Ts) allele of STS37 (an alanine-to-proline substitution at position 25) are each sufficient to convert a wild-type *RAS2* into a dominantly interfering gene.

Ability of *CDC25* overexpression to reverse the effect of the dominant-interfering *RAS2*(Ts) genes. The ability of the dominant *RAS2*$^{Val-19, Ala-22}$ alleles to block cell growth even in the presence of excess wild-type *RAS*, but not when *RAS2*$^{Val-19}$ is present, is reminiscent of the growth inhibition induced by disruption of *CDC25* (6, 38). We wished therefore to explore the possibility that the dominant *RAS2*(Ts) genes might block the activation of wild-type RAS proteins by depleting CDC25 protein. The *ras1*$^-$ *RAS2*(Ts) mutants STS13 and STS37, and their *RAS1*$^+$ counterparts RS60-3A and RS68-2B (see Materials and Methods), were transformed with the high-copy vector YEp*CDC25-1* (6). We found that like *ras1*$^-$ *ras2*(Ts) strains, the *ras1*$^-$ *RAS2*(Ts) strains did not have their temperature-sensitive defect reversed by overexpression of *CDC25*. However, the temperature-sensitive defect of the *RAS1*$^+$ *RAS2*(Ts) strains was overcome by overexpression of *CDC25* (data not shown). It would appear, then, that *CDC25* overexpression can reverse the temperature-sensitive defect induced by *RAS2*(Ts) alleles, but only in the presence of wild-type *RAS*.

TABLE 2. Evidence that suppression of the temperature-sensitive phenotype of STS13 requires both overexpression of *CDC25* and the presence of *RAS2*[a]

Presence of gene on high-copy plasmids		No. of colonies with given phenotype	
RAS2	*CDC25*	Ts$^+$	Ts$^-$
−	−	2	145
−	+	0	54
+	−	1	343
+	+	349	11

[a] Strain STS13 (*ras1*$^-$ *RAS2*$^{Val-19, Ala-22}$) was transformed with both an episomal *RAS2* plasmid (*HIS3*$^+$) and a high-copy *CDC25* plasmid (*LEU2*$^+$). After plasmid segregation (see text), the temperature-sensitive phenotype of individual colonies was scored at 35°C on YPD plates, as was the presence or absence of the two plasmids.

To confirm this finding, the *ras1*$^-$ *RAS2*$^{Val-19, Ala-22}$ strain STS13 was transformed sequentially with YEp*CDC25-1* and then with the episomal *RAS2* vector pRG2 (see Materials and Methods). Double transformants were allowed to grow in nonselective media overnight to allow for loss of either or both of the *CDC25* and *RAS2* plasmids. Individual cells were allowed to grow into colonies and then replica plated to assess the segregation of three phenotypes: temperature-sensitive growth, the presence of high-copy *CDC25* (Leu$^+$), and the presence of wild-type *RAS2* (His$^+$). The results shown in Table 2 demonstrate the requirement of both overexpression of *CDC25* and the presence of wild-type *RAS2* to overcome the temperature-sensitive block induced by *RAS2*$^{Val-19, Ala-22}$. Thus, we confirmed that overexpression of *CDC25* reverses the temperature-sensitive defect imposed by *RAS2*$^{Val-19, Ala-22}$ only in the presence of wild-type RAS protein. This result suggests to us that the *RAS2*$^{Val-22, Ala-22}$ proteins can deplete the cell of normal, endogenous levels of *CDC25* activity.

Analogous properties of H-*ras*$^{Val-12, Ala-15}$ and H-*ras*$^{Ala-15}$ mutants. Wild-type mammalian H-*ras* can suppress the growth defects induced by disruption of *CDC25* (31), whereas high expression of yeast RAS proteins does not (6, 31). This has raised the possibility that H-ras protein does not interact with CDC25 protein. We decided to reexamine this question by constructing H-*ras* mutants with amino acid substitutions analogous to the substitutions found in the dominant interfering *RAS2*(Ts) mutant genes. We used oligonucleotide site-directed mutagenesis to construct two H-*ras* genes: H-*ras*$^{Ala-15}$ and H-*ras*$^{Val-12, Ala-15}$ (see Materials and Methods). These genes were transferred into the galactose-inducible yeast expression vector YEp51 (5). We transformed the wild-type strain SP1 with YEp51 vectors

TABLE 3. Ability of overexpressed genes to override growth inhibition of H-*ras*$^{Val-12, Ala-15}$[a]

Overexpressed gene	Growth of colonies (no.) on YP + galactose	
	Ts$^-$	Ts$^+$
None	113	0
RAS2	55	2
TPK1	1	87
CDC25	2	120

[a] Strain SDR3, which expresses H-*ras*$^{Val-12, Ala-65}$ when induced with galactose (see text), was transformed with various high-copy plasmids, including YEp13, YEp*RAS2*, YEp*TPK1*, and YEp*CDC25-1* (see Materials and Methods). Four days later, the transformed colonies were replica plated onto either glucose- or galactose-containing plates and incubated at 37°C. The ability of the high-copy plasmids to restore growth at 37°C on galactose-containing plates was scored after 2 days.

expressing wild-type H-*ras*, H-*ras*$^{Val-12}$, H-*ras*$^{Ala-15}$, and H-*ras*$^{Val-12, Ala-15}$ and tested the effect of galactose-induced expression on the temperature-sensitive growth properties of the transformants. The expression of neither wild-type H-*ras* nor H-*ras*$^{Val-12}$ interfered with growth on galactose-containing medium at any temperature tested. However, expression of both H-*ras*$^{Ala-15}$ and H-*ras*$^{Val-19, Ala-15}$ blocked growth on galactose at all temperatures tested. The effect was more pronounced at higher temperatures (total growth inhibition at 35°C) and somewhat leaky at 23°C (thin patches formed after 4 days).

We tested whether various high-copy plasmid vectors containing genes of the *RAS*/cAMP pathway could reverse the effects of the mutant H-*ras* genes. Since the YEp51 vectors and our *CDC25* overexpression vectors both utilize the *LEU2* marker, we constructed *HIS3*$^+$ vectors containing H-*ras*$^{Ala-15}$ and H-*ras*$^{Val-19, Ala-15}$ (see Materials and Methods). These integration vectors were transformed into SP1 after cleavage within the *HIS3* gene by *Xho*I to site direct integration into the *HIS3* locus. Both of these integration vectors were found to block growth in the presence of galactose, although the effect was much more temperature dependent than with the high-copy episomal vectors expressing the mutant H-*ras* genes. Strain SDR-3, which expresses H-*ras*$^{Val-12, Ala-15}$ when induced with galactose, was transformed with high-copy vectors which overexpressed wild-type *RAS2*, the cAMP-dependent protein kinase gene *TPK1*, and *CDC25*. The effect of these high-copy plasmids is shown in Table 3. As expected, overexpression of *TPK1* reversed the temperature-sensitive defect. Overexpression of wild-type *RAS2* did not enhance cell growth (Table 3), indicating that the block of wild-type *RAS* function cannot be inhibited by an abundance of wild-type RAS protein. Overexpression of *CDC25* completely reverses the temperature-sensitive defect (Table 3). Thus, the H-*ras*$^{Val-12, Ala-15}$ mutant, like the dominant *RAS2*(Ts) alleles, acts to block *RAS* function by depleting the normal endogenous supply of *CDC25* activity. This result suggests that H-ras protein can interact with CDC25 protein in a manner analogous to the interaction of RAS2 protein with CDC25 protein.

DISCUSSION

We have described dominant mutations of *RAS2* and H-*ras* which interfere with wild-type *RAS* function in *S. cerevisiae*. The evidence is clear that the mutant *RAS* genes interfere with the activation of normal RAS proteins, since the interference can be overcome by expression of mutationally activated *RAS2*$^{Val-19}$. More specifically, interfering *RAS* mutants appear to block *CDC25* activity, since overexpression of CDC25 protein, which acts upstream of RAS protein and is required for its activation (6, 38), can overcome the interference if wild-type RAS protein is also present. Overexpression of *CDC25* has no apparent phenotype in an otherwise wild-type cell (6), nor does it suppress the temperature-sensitive defect of yeast cells expressing only temperature-sensitive RAS proteins. We therefore propose that interfering RAS protein forms a complex with CDC25 protein and thereby blocks its activity.

We propose that the mechanism of interaction between CDC25 and RAS proteins may be evolutionarily conserved, since expression of H-*ras*$^{Ala-15}$ seems to deplete the normal supply of CDC25 protein in *S. cerevisiae*. Moreover, Sigal and co-workers have reported that an H-*ras*$^{Asn-16}$ mutation also induces a dominant temperature-sensitive growth arrest that likewise can be reversed by the expression of an activated *RAS2* mutant gene (40). Recently, Feig and Cooper reported similar mutations in mammalian cells (14). They found that an H-*ras*$^{Asn-17}$ mutant inhibits proliferation of mouse NIH 3T3 cells and that the effect of H-*ras*$^{Asn-17}$ is reversed by expression of the activated mutant H-*ras*$^{Val-12}$ gene (14). This strongly suggests to us that a protein with the properties of the CDC25 protein also exists in mammalian cells and is required for activation of wild type H-ras protein. We have not tested the H-*ras*$^{Asn-17}$ mutant in yeast cells or the H-*ras*$^{Ala-15}$ mutant in mammalian cells.

The amino acid substitutions occurring in the dominant RAS2^{ts} proteins are located in a highly conserved guanine-nucleotide-binding region (Table 4). The mutation found by Feig and Cooper is located in this region (14), as is the mutant H-*ras* described by Sigal and co-workers (40). The *RAS*-like gene products, such as *RHO*, *YPT*, and *SEC4*, and G proteins, such as $G_s\alpha$, $G_i\alpha$, and transducins, have conserved residues corresponding to residues 10 to 18 of the human H-*ras* protein and virtual identity at positions which correspond to residues 15, 16, and 17 (18, 21, 23, 28–30, 34, 39, 42). This region corresponds to a consensus nucleotide-

TABLE 4. Partial amino acid sequence of RAS and RAS-like proteins in region of interfering mutant proteins[a]

Protein	Sequence	Positions	Reference(s)
	↓ ↓ ↓ ↓		
H-ras	**GLY ALA GLY GLY VAL GLY LYS SER ALA**	10–18	13
RAS1,2	**GLY GLY GLY GLY VAL GLY LYS SER ALA**	17–25	35
YPT1	**GLY ASN SER GLY VAL GLY LYS SER CYS**	15–23	18, 21
SEC4	**GLY ASP SER GLY VAL GLY LYS SER CYS**	27–35	39
Transducin	**GLY ALA GLY GLY SER GLY LYS SER THR**	40–48	28, 34

[a] The region of H-ras and other related proteins where the mutations of the dominant-interfering genes are localized includes a highly conserved GXXXXGK sequence (in boldface) found in virtually all guanine-nucleotide-binding proteins (12). Arrows indicate positions where amino acid substitutions within H-ras or RAS2 proteins lead to proteins with interfering properties.

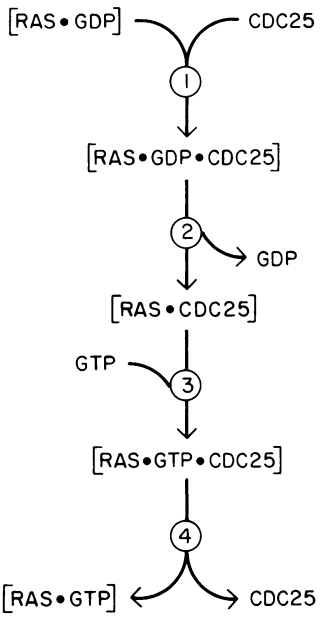

FIG. 1. Model for RAS and CDC25 protein interactions. In step 1, RAS complexed to GDP binds to CDC25 protein. In step 2, CDC25 stabilizes the nucleotide-free state of RAS, and GDP dissociates. In step 3, GTP binds to RAS. This terminates the interaction, and in step 4 CDC25 protein is free to interact with another RAS protein. According to this model, the dominant interfering RAS proteins cannot proceed through steps 3 and 4 and instead remain bound to CDC25, preventing it from interacting with wild-type RAS protein.

binding sequence, GXXXXGK (Table 4). The interfering mutant H-ras proteins of Feig and Cooper and of Sigal and co-workers both exhibit altered affinity for guanine nucleotides (14, 40). This suggests that alterations in guanine-nucleotide-binding properties underlie the ability of the mutant RAS proteins to deplete *CDC25* activity in cells.

The results presented in this report lead us to propose a simple model for the inhibition of wild-type *RAS* function by the dominant *RAS2*(Ts) mutants (Fig. 1). We propose that RAS and CDC25 proteins normally undergo a transient, direct interaction, similar to the transient interaction of receptors with G proteins (20, 41). In this model, CDC25 proteins interact with the GDP-bound form of RAS proteins and, by virtue of stabilizing the transitional state of nucleotide-free RAS protein, catalyze nucleotide exchange. This mechanism of catalysis (stabilization of the nucleotide-free transitional state) has been proposed by Stryer for the activation of transducin by photorhodopsin (41). When GTP binds to RAS protein, the interaction is terminated and CDC25 protein is free to interact with another RAS protein. We propose that the dominant-interfering RAS proteins remain bound to CDC25 protein, because either a severely impaired ability to bind guanine nucleotides or a selective inability to bind GTP results in a failure to terminate the interaction with CDC25 protein. The net result of the interaction between CDC25 and interfering RAS proteins is to prevent CDC25 protein from interacting with wild-type RAS proteins. This causes a *cdc25*-like state, consistent with the genetic experiments we described.

We envision that mutants in other *RAS*-like genes, similar to the dominant-interfering mutants we have described, may aid in identification of their respective *CDC25*-like gene product. The ability of overexpression of *CDC25* to suppress the dominant *RAS2*(Ts) mutations only in the presence of wild-type *RAS* forms the basis for a highly selective genetic screen. We also envision that mutant *RAS* genes encoding proteins blocked in other steps of the RAS protein cycle can be identified and utilized for the study of other proteins with which RAS proteins interact. In support of this, an H-*ras* mutant capable of interfering with the function of $RAS2^{Val-19}$ has been isolated and characterized recently (T. Michaeli, et al., unpublished data). The existence of interfering mutants in signal transduction pathways may be quite general (22). For example, we have found dominant-interfering forms of adenylate cyclase in yeast cells which appear to block *RAS* function (J. Field et al., unpublished data). Analogous mutants may exist for the targets of H-ras proteins in mammalian cells. We have seen that mutant oncogene products may interfere with cell growth. As a further generalization, we may be able to generate interfering mutants of "recessive" oncogenes, such as the retinoblastoma gene (9, 17), which thereby become dominant oncogenes.

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