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The *Saccharomyces cerevisiae* SDC25 C-Domain Gene Product Overcomes the Dominant Inhibitory Activity of Ha-Ras Asn-17

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The carboxy-terminal part of the *Saccharomyces cerevisiae SDC25* gene product (SDC25 C domain) can elicit activation of mammalian Ras proteins. Specifically, SDC25 C domain functions as an exchange factor for cellular Ras proteins in CHO cells. In this study, we used the dominant inhibitory Ha-Ras Asn-17 mutant and SDC25 C domain to further investigate the interaction between cellular Ras proteins and their putative endogenous guanine nucleotide-releasing factors. Transcription from the polyomavirus thymidine kinase gene (Py *tk*) promoter is strongly inhibited by the expression of Ha-Ras Asn-17 in NIH 3T3 cells. Coexpression of SDC25 C domain overcomes the negative effect of the Ras mutant on the Py *tk* promoter. On the other hand, transactivation of the Ras-responsive element of the Py *tk* promoter induced by SDC25 C domain is lost upon coexpression of increasing amounts of Ha-Ras Asn-17. In addition, coexpression of SDC25 C domain overcomes the inhibition of proliferation of NIH 3T3 cells caused by Ha-Ras Asn-17. These results are consistent with the idea that the Ha-Ras Asn-17 mutant functions by titrating an upstream activator of cellular Ras proteins.

The identification of intermolecular interactions required for Ras protein activity would greatly facilitate the analysis of their role in signal transduction. Of the proteins known to functionally interact with Ras, the best characterized is GTPase-activating protein (GAP) (37). GAP stimulates the conversion of Ras-GTP to Ras-GDP, thereby downregulating the biological function of Ras proteins. GAP interacts with Ras at the effector binding domain (1, 10, 28) and may act as an effector as well as a negative regulator (23, 31, 42). Another set of recent studies suggests that the catalytic fragment of the neurofibromatosis gene might function similarly to GAP (2, 22). That critical interactions occur between cellular Ras proteins and upstream regulators has also been suggested, although such interactions have not yet been characterized. A set of guanine nucleotide-releasing factors (GRFs), which stimulate the dissociation of GDP from Ras proteins in vitro, was recently partially purified (39, 40). Another protein, called Smg p21-GDS, was recently identified, and its gene was cloned. Smg p21-GDS facilitates GDP dissociation from the small GTP-binding proteins Rap1a, Rap1b, and RhoA and from posttranslationally modified Ki-Ras, but not from Ha-Ras or N-Ras (20, 24). In *Saccharomyces cerevisiae*, this function has been ascribed to the product of the *CDC25* gene (6). *CDC25* and the 3′ encoded region of *SDC25* (for suppressor of the *CDC25.5* mutation) appear to be involved in activating yeast Ras (5). Recently the cloning of mammalian homologs of CDC25 was reported (4, 21, 32). The carboxy-terminal domain of SDC25 (SDC25 C domain) was also shown to facilitate GDP-for-GTP exchange on mammalian Ras proteins in vitro (12) and in CHO cells (27). Stable expression of the SDC25 C domain induces transformation of NIH 3T3 cells, probably by favoring GTP loading onto cellular Ras proteins (3).

Stimulation of different NIH 3T3 cell types with epidermal growth factor, platelet-derived growth factor, insulin, or other growth factors (7, 9, 19, 29, 30) rapidly activates Ras proteins. However, a direct interaction between these growth factor receptors and Ras has not been shown. To explain such an activation, at least two different mechanisms can be proposed: the deactivating function of GAP may be downregulated after growth factor receptors induce tyrosine phosphorylation (14, 15, 25), or a GRF molecule may be activated. To unravel such issues, the use of dominant negative mutants of Ras proteins might be useful. Among these mutants, Ha-Ras Asn-17, in which Ser-17 has been changed to Asn, inhibits proliferation of NIH 3T3 cells (9, 18) and neuronal differentiation of PC12 cells (34). The growth-inhibitory effect of Ha-Ras Asn-17 can be bypassed by expression of the *raf* or *mos* oncogene, both of which function downstream or independently of cellular Ras proteins (18). In *S. cerevisiae*, a neighboring mutation in yeast *RAS2* confers dominant inhibitory properties to this gene (26). The inhibitory property of this gene product could be overcome by overexpression of CDC25 in the presence of wild-type *RAS2* (26). Thus, a likely explanation for such an inhibitory function was that Ha-Ras Asn-17 acts as a competitive antagonist of an endogenous Ras activator (9, 16).

The objective of this study was to determine whether the dominant negative mutant Ha-Ras Asn-17 disrupts a critical interaction between cellular Ras and a mammalian GRF. Ha-Ras Asn-17 titrated the functional activity of SDC25 C domain, as measured by the transactivation of the polyomavirus (Py) enhancer. In addition, SDC25 C domain, which functions as a GRF in CHO cells (27) and transforms NIH 3T3 cells (3), overcomes the growth-inhibitory activity of Ha-Ras Asn-17.

MATERIALS AND METHODS

Expression vectors. The DNA fragment encoding SDC25 C domain, starting at the penultimate *Bam*HI site up to the *Bcl*I

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site after the open reading frame, has been cloned under the control of the simian virus 40 early promoter in the pcym1 vector (27). This expression vector is designated pRG17. As a control, the vector pRG17ΔBam, carrying a deletion internal to the SDC25 C domain, was constructed. This deletion was obtained by removal of the *Bam*HI-*Bam*HI fragment within the open reading frame of *SDC25* (5).

The Ha-Ras Asn-17 mutant was the result of site-directed mutagenesis on the Ha-Ras human cDNA (18). This mutant cDNA was placed under the control of the simian virus 40 early promoter (pSV2 Asn^{17}) for use in transient expression assays.

For stable expression experiments, the pZIP vector was used, with resistance to neomycin as the dominant selection marker (18). A GAP expression vector (pSV2 GAP) was constructed by inserting the human GAP cDNA sequence into the pSV2 vector (31). For transient transfection experiments, we used the chloramphenicol acetyltransferase (CAT) gene under the control of a synthetic promoter containing four repeats of the PEA1 motif derived from the Py enhancer (31).

Transient transfections. NIH 3T3 cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (FCS) at 37°C in the presence of 5% CO_2. Cells that were 50% confluent were transfected for 4 h in serum-free medium, using Transfectam (Sepracor-IBF) as the transfection reagent. For each experimental point, 0.5 μg of CAT plasmid and a constant amount of DNA bearing the same amount of promoter were used. The cells were collected 48 h after transfection, and CAT activity was measured. The assays were quantified by cutting out spots containing the acetylated forms of [^{14}C]chloramphenicol (Amersham).

Stably transfected cell lines. NIH 3T3 cells were transfected with plasmid DNA as previously described (18). Three days after transfection, cells were subcultured into medium containing 400 μg of G418 per ml. Two weeks later, neomycin-resistant colonies were either stained and counted or isolated for further analysis.

Northern (RNA) blot analysis. Total cytoplasmic RNA was isolated and electrophoresed in 1% agarose–formaldehyde gels as described previously (9). Gels were transferred to GeneScreen Plus filters (Dupont) and hybridized with a ^{32}P-labeled human Ha-Ras probe.

Immunoblot analysis. The peptide corresponding to the 61–80 region of SDC25 C domain, GYGSYDGGETEKS DTNAVYA, was coupled via an additional N-terminal cysteine to keyhole limpet hemocyanin, using succinimidyl 4-(*p*-maleimidophenyl) butyrate. Rabbit antibody titers against the keyhole limpet hemocyanin-peptide fusion were measured by enzyme-linked immunosorbent assay. Anti-SDC25 C domain was purified by passage of the serum on a protein A-Sepharose column (Pharmacia) followed by 61–80 peptide affinity chromatography.

RESULTS

SDC25 C domain and Ha-Ras Asn-17 have antagonistic effects on transactivation of the Ras-responsive element PEA1. We initially compared the effects of SDC25 C domain and Ha-Ras Asn-17 on expression of a Ras-responsive element of the Py enhancer. A plasmid containing four head-to-tail copies of the PEA1 motif upstream of the thymidine kinase gene (*tk*)-CAT fusion sequence was used to study the functional interaction between Ha-Ras Asn-17 and SDC25 C domain in NIH 3T3 cells. SDC25 C domain induced CAT activity in serum-starved cells (Fig. 1). In contrast, Ha-Ras Asn-17 completely abolished the CAT activity induced by serum stimulation of the cells (Fig. 1). Thus, SDC25 C domain and Ha-Ras Asn-17 had opposite effects on the Py enhancer, consistent with their acting as positive and negative regulators of the same signaling pathway. To determine whether the Ha-Ras Asn-17 mutant blocked an upstream regulator of cellular Ras, we tested whether it would decrease the activity of SDC25 C domain in NIH 3T3 cells.

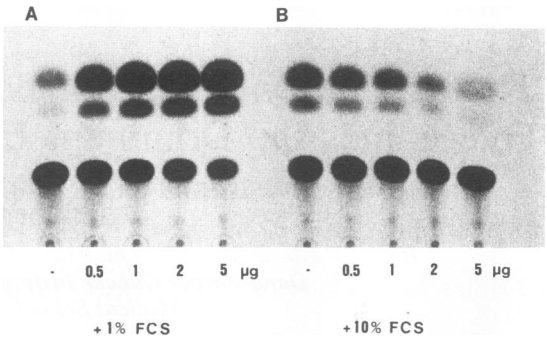

FIG. 1. CAT activity in NIH 3T3 cells induced by SDC25 C domain and Ha-Ras Asn-17. (A) Cells were cotransfected with 0.5 μg of a vector containing the CAT gene under the control of a Py *tk* promoter and with increasing amounts of pRG17. Total DNA was adjusted to 5 μg with an expression vector without an insert. Cells were grown after 4 h of transfection in 1% FCS. (B) Py *tk*-CAT was transfected along with increasing amounts of pSV2 Asn^{17}; after transfection, cells were grown in 10% FCS to induce CAT expression.

Induction of CAT expression by SDC25 C domain was completely blocked by increasing the amount of Ha-Ras Asn-17 (Fig. 2A). We then tested whether, alternatively, SDC25 C domain could overcome the transcriptional block to the Py *tk* promoter caused by Ha-Ras Asn-17. As expected, transfection of increasing amounts of SDC25 C

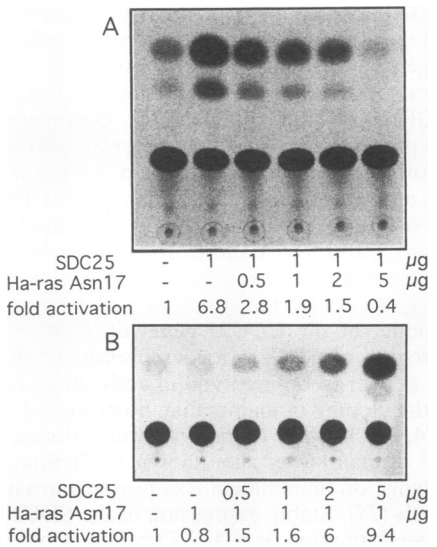

FIG. 2. Antagonistic effects of Ha-Ras Asn-17 and SDC25 C domain on CAT expression. Cells were cultured in 1% FCS after transfection with 1 μg of pRG17 and increasing amounts of pSV2 Asn^{17} (A) or 1 μg of pSV2 Asn^{17} and increasing amounts of pRG17 (B).

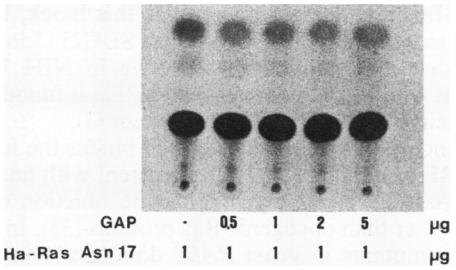

FIG. 3. Effect of Ha-ras Asn-17 (1 μg of pSV2 Asn^{17}) on CAT expression in the presence of increasing amounts of Ras-GAP expression vector pSV2 GAP. Cells were grown in 1% FCS after transfection.

domain resulted in renewed CAT transcription (Fig. 2B). Thus, it appeared that the negative effect exerted by Ha-Ras Asn-17 on CAT transcription had been reversed by a guanine nucleotide exchange factor. As a control, Ha-Ras Asn-17 function was not overcome by the overexpression of GAP, a putative downstream effector for Ras (Fig. 3).

Growth inhibition by Ha-Ras Asn-17 can be overcome by coexpression of SDC25 C domain. Whether SDC25 C domain would overcome the growth inhibition of Ha-Ras Asn-17 was investigated by cotransfection assays in NIH 3T3 cells. Cells were transfected with a neomycin resistance gene either alone [the pZIPneoSV(x) vector] or linked to Ha-Ras Asn-17 (plasmid pZIP M17) (Fig. 4). As previously observed (18), about 10-fold fewer neomycin-resistant colonies were obtained from pZIP M17-transfected plates than from those transfected with pZIPneoSV(X), indicating that Ha-Ras Asn-17 had inhibited the proliferation of transfected cells. In contrast, cotransfection with pZIP M17 plus SDC25 C domain yielded a number of neomycin-resistant colonies similar to that obtained with pZIPneoSV(X). As a control, cotransfection with the inactive SDC25 C-domain mutant failed to overcome the growth-inhibitory effect of Ha-Ras Asn-17. These results indicate that the functional SDC25 C domain can reverse the inhibition of cell proliferation resulting from Ha-Ras Asn-17 expression.

To further test the ability of SDC25 C domain to overcome the growth inhibition due to Ha-Ras Asn-17, expression of the mutant *ras* gene in the neomycin-resistant transfectants was investigated by Northern blotting (Fig. 5). Five neomycin-resistant cell lines obtained from cotransfection with pZIP M17 plus SDC25 C domain all expressed 5.6-kb Ha-Ras transcripts, as expected for transcription of Ha-Ras Asn-17 in the pZIP vector (18). In contrast, no expression of the Ha-Ras Asn-17 gene was detected in the rare neomycin-resistant cell lines isolated after transfection with pZIP M17 alone or after cotransfection with the inactive SDC25 C-domain mutant. Immunoblot analysis of derivatives of NIH 3T3 cells with anti-SDC25 C-domain polyclonal antibodies confirmed expression of SDC25 C domain in the stable transfectants. Cell lines transfected with Ha-Ras Asn-17 and SDC25 C domain expressed detectable levels of an exogenous protein in the size range of 60 kDa (Fig. 5).

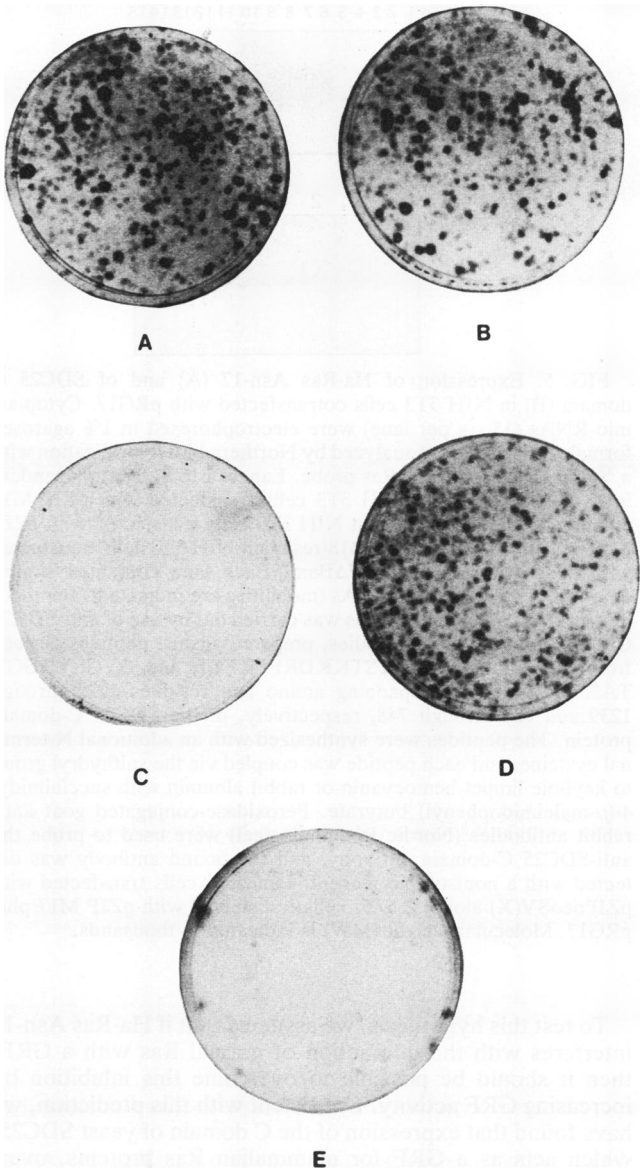

FIG. 4. Cotransfection of NIH 3T3 cells with Ha-Ras Asn-17 and SCD25. NIH 3T3 cells were transfected with plasmid DNAs and subcultured into medium containing G418. All transfections were with 0.1 μg of pZIPneoSV(X) or pZIP M17 and 0.5 μg of pRG17 or pRG17ΔBam. Colonies were stained and photographed 17 days after transfection. (A) pZIPneoSV(X); B, pZIPneoSV(X) plus pRG17; C, pZIP M17; D, pZIP M17 plus pRG17; E, pZIP M17 plus pRG17ΔBam.

DISCUSSION

In this study, we investigated the relationship between the dominant inhibitory mutant Ha-Ras Asn-17 and a critical step in Ras activation, the requirement for a GRF. A priori, disruption of the normal interactions of Ras with either an upstream regulator (e.g., GRF) or a downstream effector (e.g., GAP or other effector proteins) could be envisioned to explain the dominant inhibitory activity of Ha-Ras Asn-17. However, Ha-Ras Asn-17 has a preferential affinity for GDP versus GTP (18), suggesting that the active inhibitory protein is in the GDP-bound state, which would not be expected to bind to GAP (37). In addition, mutations in the effector (and GAP-binding) domain of Ha-Ras Asn-17 do not affect its inhibitory activity (33). It thus appears unlikely that an effector molecule or GAP represents the target for inhibition by Ha-Ras Asn-17, favoring the hypothesis that this mutant protein may interfere with the interaction between normal Ras and a GRF required for Ras activation.

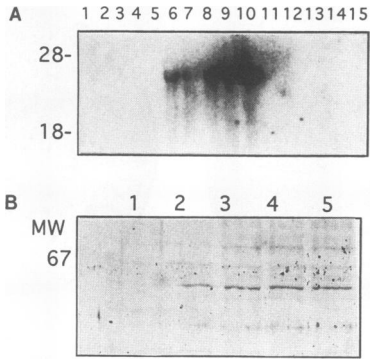

FIG. 5. Expression of Ha-Ras Asn-17 (A) and of SDC25 C domain (B) in NIH 3T3 cells cotransfected with pRG17. Cytoplasmic RNAs (15 μg per lane) were electrophoresed in 1% agarose–formaldehyde gels and analyzed by Northern blot hybridization with a ^{32}P-labeled human Ha-Ras probe. Lanes: 1 to 5, five independent lines of G418-resistant NIH 3T3 cells transfected with pZIP M17 alone; 6 to 10, G418-resistant NIH 3T3 cells transfected with pZIP M17 plus pRG17; 11 to 15, G418-resistant NIH 3T3 cells transfected with pZIP M17 plus pRG17ΔBam. Each lane contained similar amounts of 18S and 28S rRNAs (mobilities are indicated). Immunoblot analysis of the cell lysates was carried out by use of anti-SDC25 C-domain polyclonal antibodies, prepared against peptides derived from the sequences GKTSTKKDRFPKFQL and GYGSYDGGE TAEKSDTNAVYA, spanning amino and residues 1225 through 1239 and 728 through 748, respectively, of the SDC25 C-domain protein. The peptides were synthesized with an additional N-terminal cysteine, and each peptide was coupled via the sulfhydryl group to keyhole limpet hemocyanin or rabbit albumin with succinimidyl 4-(*p*-maleimidophenyl) butyrate. Peroxidase-conjugated goat anti-rabbit antibodies (Nordic Immunological) were used to probe the anti-SDC25 C-domain antibody, and the bound antibody was detected with a nonisotopic reagent. Lanes: 1, cells transfected with pZIPneoSV(X) alone; 2 to 5, cells transfected with pZIP M17 plus pRG17. Molecular weight (MW) is indicated in thousands.

To test this hypothesis, we assumed that if Ha-Ras Asn-17 interferes with the interaction of normal Ras with a GRF, then it should be possible to overcome this inhibition by increasing GRF activity. Consistent with this prediction, we have found that expression of the C domain of yeast SDC25, which acts as a GRF for mammalian Ras proteins, overcomes the dominant inhibitory activity of Ha-Ras Asn-17 in both stable transformation and transient expression assays. Thus, cotransfection with a plasmid expressing SDC25 C domain reversed the inhibitory effect of Ha-Ras Asn-17 on NIH 3T3 cell proliferation. Similarly, expression of SDC25 C domain reversed the inhibitory effect of Ha-Ras Asn-17 expression on activation of the Py enhancer in response to serum stimulation.

The ability of SDC25 C domain to overcome the inhibitory activity of Ha-Ras Asn-17 was, in principle, consistent with the possibility that SDC25 C domain acts downstream or independently of Ras, rather than overcoming a deficiency in GRF activity resulting from expression of the mutant Ras protein. However, we also found that Ha-Ras Asn-17 inhibited activation of the Py enhancer by SDC25 C domain in serum-starved cells. This result confirms that SDC25 C domain acts upstream of normal Ras proteins, as expected from its activity as a Ras-GRF. The ability of SDC25 C domain to also overcome the inhibitory activity of Ha-Ras Asn-17 therefore suggests that the dominant inhibitory activity of Ha-Ras Asn-17 arises from competition with normal Ras for a GRF in mammalian cells. Sufficiently high expression of SDC25 C domain overcomes this block, leading to normal Ras activation. This activity of SDC25 C domain may suggest that it is constitutively active in NIH 3T3 cells, consistent with recent data suggesting that it functions as an oncoprotein in the NIH 3T3 cell system (3).

The conclusion that Ha-Ras Asn-17 blocks the function of a Ras-GRF in NIH 3T3 cells is consistent with findings that Ha-Ras Asn-17 selectively inhibits the function of normal cellular rather than oncogenic Ras proteins (33). In addition, analogous mutants in yeast *RAS2* dominantly inhibit RAS function in *Saccharomyces cerevisiae*, and the inhibitory effects of these mutants can be overcome by overexpression of CDC25 (26).

The results presented above indicate that mammalian exchange factors of the CDC25 class may constitute a vital upstream regulator of Ras, serving as a link between tyrosine kinase growth factor receptors and cellular Ras activation. Mammalian CDC25 homologs so far identified do not contain sequence elements known to interact with protein-tyrosine kinases (Src homology region SH2 or SH3) (4, 20, 21, 32). This finding suggests that mammalian GRF may not directly associate with tyrosine kinases but may instead be activated via an adaptor molecule such as SEM5, which has been identified in *Caenorhabditis elegans* (11). The inhibitory activity of Ha-Ras Asn-17 has proven useful in a number of studies directed toward elucidating the relationships between Ras and candidate effectors that may act downstream of Ras in intracellular signal transduction pathways (8, 13, 35, 36, 38, 41). The inhibitory effect of Ha-Ras Asn-17 on the normal interactions of Ras with a GRF may likewise help to elucidate the upstream pathways leading to Ras activation in response to growth factors.

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F.S. and H.C. contributed equally to this study.

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REFERENCES

1. **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.
2. **Ballester, R., D. Marchuk, M. Boguski, A. Saulino, R. Letcher, M. Wigler, and F. Collins.** 1990. The NF1 locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell **63**:851–859.
3. **Barlat, I., F. Schweighoffer, M. C. Chevallier-Multon, M. Duchesne, I. Fath, D. Landais, M. Jacquet, and B. Tocque.** The Saccharomyces cerevisiae gene product SDC25 C-domain functions as an oncoprotein in NIH 3T3 cells. Oncogene, in press.
4. **Bowtel, D., P. Fu, M. Simon, and P. Senior.** 1992. Identification of murine homologues of the Drosophila son of sevenless gene: potential activators of ras. Proc. Natl. Acad. Sci. USA **89**:6511–6515.
5. **Boy-Marcotte, E., F. Damak, J. Camonis, H. Garreau, and M. Jacquet.** 1989. The C-terminal domain of a gene partially homologous to CDC25 gene suppresses the cdc25-5 mutation in *Saccharomyces cerevisiae*. Gene **77**:21–30.
6. **Broek, D., T. Toda, T. Michaeli, C. 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.
7. **Burgering, B. M. T., R. H. Medena, J. A. Maasen, M. L. Van de Wetering, A. J. Van der Eb, F. McCormick, and J. L. Bos.** 1991. Insulin stimulation of gene expression mediated by p21ras

activation. EMBO J. **10**:1103–1109.
8. **Cai, H., P. Erhardt, J. Szeberenyi, M. T. Diaz-Meco, J. Moscat, and G. M. Cooper.** Hydrolysis of phosphatidylcholine is stimulated by ras proteins during mitogenic signal transduction. Mol. Cell. Biol. **12**:5329–5335.
9. **Cai, H., J. Szeberenyi, and G. M. Cooper.** 1990. Effect of dominant inhibitory Ha-*ras* mutation on mitogenic signal transduction in NIH 3T3 cells. Mol. Cell. Biol. **10**:5314–5323.
10. **Calès, 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.
11. **Clark, S. G., M. J. Stern, and R. Horvitz.** 1992. C. elegans cell-signalling gene *sem-5* encodes a protein with SH2 and SH3 domains. Nature (London) **256**:340–344.
12. **Crèchet, 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.
13. **De Vries-Smits, A. M. M., B. M. T. Burgering, S. J. Leevers, C. J. Marshall, and J. L. Bos.** 1992. Involvement of p21 ras in activation of extracellular signal-regulated kinase 2. Nature (London) **357**:602–604.
14. **Downward, J., J. D. Graves, P. H. Warne, S. Rayter, and D. A. Cantrell.** 1990. Stimulation of $p21^{ras}$ upon T cell activation. Nature (London) **346**:719–723.
15. **Ellis, C., M. Moran, F. McCormick, and T. Dawson.** 1990. Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. Nature (London) **343**: 377–381.
16. **Farnsworth, C. L., and L. A. Feig.** 1991. Dominant inhibitory mutations in the Mg^{2+}-binding site of Ras prevent its activation by GTP. Mol. Cell. Biol. **11**:4822–4829.
17. **Feig, L. A., and G. M. Cooper.** 1988. Relationship among guanine nucleotide exchange, GTP hydrolysis, and transforming potential of mutated Ras proteins. Mol. Cell. Biol. **8**:2472–2478.
18. **Feig, L. A., and G. M. Cooper.** 1988. Inhibition of NIH 3T3 cell proliferation by a mutant Ras protein with preferential affinity for GDP. Mol. Cell. Biol. **8**:3235–3243.
19. **Gibbs, J. B., M. S. Marshall, E. M. Scolnick, R. A. F. Dixon, and U. S. Vogel.** 1990. Modulation of guanine nucleotides bound to Ras in NIH 3T3 cells by oncogenes, growth factors, and the GTPase activating protein (GAP). J. Biol. Chem. **265**:20437–20442.
20. **Kaibuchi, K., T. Mizuno, H. Fujioka, T. Yamamoto, K. Kishi, Y. Fukumoto, Y. Hori, and Y. Takai.** 1991. Molecular cloning of the cDNA for stimulatory GDP/GTP exchange protein for *smg* p21s (*ras* p21-like small GTP-binding proteins) and characterization of stimulatory GDP/GTP exchange protein. Mol. Cell. Biol. **11**:2873–2880.
21. **Martegani, E., M. Vanoni, R. Zippel, P. Coccetti, R. Brambilla, C. Ferrari, E. Sturani, and L. Alberghina.** 1992. Cloning by functional complementation of a mouse cDNA encoding a homologue of CDC25, a *Saccharomyces cerevisiae* RAS activator. EMBO J. **11**:2151–2157.
22. **Martin, G. A., D. Viskochil, G. Bollag, P. C. McCabe, W. J. Crosier, H. Haubruck, L. Conroy, R. Clark, P. O'Connell, R. M. Cawthon, M. A. Innis, and F. McCormick.** 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p12. Cell **63**:843–849.
23. **Martin, G. A., A. Yatani, R. Clark, L. Conroy, P. Polakis, A. M. Brown, and F. McCormick.** 1992. GAP domains responsible for Ras p21-dependent inhibition of muscarinic atrial K^+ channel currents. Science **255**:192–194.
24. **Mizuno, T., K. Kaibuchi, T. Yamamoto, M. Kawamura, T. Sakoda, H. Fujioka, Y. Matsuura, and Y. Takai.** 1991. A stimulatory GDP/GTP exchange protein for smg p21 is active on the post-translationally processed form of c-Ki-ras p21 and rhoA p21. Proc. Natl. Acad. Sci. USA **88**:6442–6446.
25. **Molloy, C. J., D. P. Bottaro, T. P. Fleming, M. S. Marshall, J. B. Gibbs, and S. A. Aaronson.** 1989. PDGF induction of tyrosine phosphorylation of GTPase activating protein. Nature (London) **342**:711–714.
26. **Powers, S., K. O'Neill, and M. Wigler.** 1989. Dominant yeast and mammalian *RAS* mutants that interfere with the *CDC25*-dependent activation of wild-type *RAS* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**:390–395.
27. **Rey, I., F. Schweighoffer, I. Barlat, J. Camonis, E. Boy-Marcotte, R. Guilbaud, M. Jacquet, and B. Tocqué.** 1991. The COOH-domain of the product of the Saccharomyces cerevisiae SCD25 gene elicits activation of p21-ras proteins in mammalian cells. Oncogene **6**:347–349.
28. **Rey, I., P. Soubigou, L. Debussche, C. David, A. Morgat, P. E. Bost, J. F. Mayaux, and B. Tocqué.** 1989. Antibodies to synthetic peptide from the residue 33 to 42 domain of c-Ha-*ras* p21 block reconstitution of the protein with different effectors. Mol. Cell. Biol. **9**:3904–3910.
29. **Satoh, T., M. Endo, M. Nakafuku, S. Nakamura, and Y. Kaziro.** 1990. Platelet derived growth factor stimulates formation of active $p21^{ras}$ GTP complex in Swiss mouse 3T3 cells. Proc. Natl. Acad. Sci. USA **87**:5993–5997.
30. **Satoh, T., M. Nakafuku, A. Miyajima, and Y. Kaziro.** 1991. Involvement of ras p21 protein in signal-transduction pathways from interleukin 2, interleukin 3, and granulocyte/macrophage colony-stimulating factor, but not from interleukin 4. Proc. Natl. Acad. Sci. USA **88**:3314–3318.
31. **Schweighoffer, F., I. Barlat, M. C. Chevallier-Multon, and B. Tocque.** 1992. Implication of Gap in Ras-dependent transactivation of a polyoma enhancer sequence. Science **256**:825–827.
32. **Shou, C., C. L. Farnsworth, B. G. Neel, and L. Feig.** 1992. Molecular cloning of cDNAs encoding a guanine-nucleotide releasing factor for Ras p21. Nature (London) **358**:351–354.
33. **Stacey, D. W., L. A. Feig, and J. B. Gibbs.** 1991. Dominant inhibitory Ras mutants selectively inhibit the activity of either cellular or oncogenic Ras. Mol. Cell. Biol. **11**:4053–4064.
34. **Szeberenyi, J., H. Cai, and G. M. Cooper.** 1990. Effect of a dominant inhibitory Ha-*ras* mutation on neuronal differentiation of PC12 cells. Mol. Cell. Biol. **10**:5324–5332.
35. **Szeberenyi, J., P. Erhardt, H. Cai, and G. M. Cooper.** Role of Ras in signal transduction from the NGF receptor: relationship to protein kinase C, calcium, and cyclic AMP. Oncogene, in press.
36. **Thomas, S. M., M. DeMarco, G. D'Arcangelo, S. Halegoua, and J. Brugge.** 1992. Ras is essential for nerve growth factor- and phorbol ester-induced tyrosine phosphorylation of MAP kinases. Cell **68**:1031–1040.
37. **Trahey, M., and F. McCormick.** 1987. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. Science **238**:542–545.
38. **Troppmair, J., J. T. Bruder, J. App, H. Cai, L. Liptak, J. Szeberenyi, G. M. Cooper, and U. R. Rapp.** 1992. Ras controls coupling of growth factor receptors and protein kinase C in the membrane to Raf-1 and B-Raf protein serine kinases in the cytosol. Oncogene **7**:1867–1873.
39. **West, M., H. F. Kung, and T. Kamata.** 1990. A novel membrane factor stimulates guanine nucleotide exchange reaction of ras proteins. FEBS Lett. **259**:245–248.
40. **Wolfman, A., and I. G. Macara.** 1990. A cytosolic protein catalyzes the release of GDP from $p21^{ras}$. Science **248**:67–69.
41. **Wood, K. W., C. Sarnecki, T. M. Roberts, and J. Blenis.** 1992. Ras mediates nerve growth factor receptor modulation of three signal-transducing protein kinases: MAP kinase, Raf-1, and RSK. Cell **68**:1041–1050.
42. **Yatani, A., K. Okabe, P. Polakis, R. Halenbeck, F. McCormick, and A. M. Brown.** 1990. Ras p21 and GAP inhibit coupling of muscarinic receptors to atrial K^+ channels. Cell **61**:769–776.