The Formin/Diaphanous-Related Protein, FHOS, Interacts with Rac1 and Activates Transcription from the Serum Response Element

Running Title: FHOS Interacts with Rac1 and Activates the SRE

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SUMMARY

FHOS is a member of the Formin homology (FH) family of proteins and is expressed at high levels in splenic cells. FH proteins link cellular signaling pathways to the actin cytoskeleton and serum response factor-dependent transcription. In these studies, the role of FHOS in Rho family GTPase signaling pathways was analyzed. FHOS interacted with the polybasic domain in the Rac1 C-terminus in a guanine nucleotide-independent manner, but did not interact with RhoA, Cdc42Hs, Rac2 or Rac3. Intramolecular autoinhibitory interactions between the C-terminus of FHOS and an N-terminal region partially overlapping the Rac1-interaction domain were also identified. FHOS truncation mutants lacking the N- or C-terminal autoregulatory domains stimulated transcription of a c-fos serum response element (SRE)-driven reporter. Overexpression of wild-type and mutant (N17 and V12) Rac1 proteins repressed SRE induction by the N-terminal FHOS deletion mutant but not by the C-terminal FHOS deletion mutant. Immunofluorescence studies indicated that the localization of the mutant FHOS proteins might contribute to their differential responses to Rac1. Wild-type FHOS and the N-terminal deletion mutant localized to the perinuclear region and membrane edges. In contrast, the C-terminal FHOS mutants were diffusely localized. These data suggest that FHOS induces transcription from SREs by multiple pathways, and that Rac1 may influence the course of some FHOS-induced signaling events.
INTRODUCTION

Formin homology (FH) proteins are highly structured proteins and components of Rho family GTPase signaling pathways that affect cytoskeletal organization and induce transcriptional activation of the serum response element (SRE) (1-6). Eukaryotic FH proteins are characterized by several conserved domains that are organized in a precise order. From the N-terminus, FH proteins contain a loosely conserved FH3 domain, a GTPase binding domain (GBD), highly conserved FH1 and FH2 domains, a coiled-coil and an autoregulatory domain (1,2,7-9). In a subset of FH proteins, the Diaphanous-Related Formin (DRF) proteins, the autoregulatory domain forms intramolecular interactions with residues within and/or between the FH3 and GBD (3). This self-interaction(s) is predicted to mask the conserved FH1 and FH2 domains, which associate with kinase signaling pathways, actin-binding proteins and microtubules (2,4,6,10-14). Inhibition of the intramolecular interactions by deleting the GBD or autoregulatory domain, or by overexpression of these domains creates constitutively active DRFs which stimulate cytoskeletal reorganization and SRF-gene transcription (3,5,6,9).

Regulation of FH protein activity is crucial for development, cell survival, cytokinesis, cell motility, and cell polarity in eukaryotes (1,3,15). Genetic mutations that alter the C-termini of mammalian FH proteins, Formin (Fmn), DRF1 (mDia1, DFNA1), and DRF2 (mDia2, DIA156) are linked to murine limb deformities, renal failure, inherited nonsyndromic deafness and premature ovarian failure in humans, respectively (16-20).

DRF proteins are activated in vivo by Rho family GTPases. Rho-related proteins are a subset of the Ras superfamily of GTPases and include RhoA-E, Rho6, Rho7, Rac1-3, Cdc42, TC10, and TTF. Rho GTPases regulate numerous cellular events, including gene transcription,
cell cycle progression, adhesion, actin cytoskeletal organization, cytokinesis and motility, by cycling between inactive GDP- and active GTP-bound states and relaying signals from membrane receptors to downstream effector molecules (21,22). DRF proteins are downstream effectors of Rho GTPases. Activated Rho GTPases bind to the GBDs of DRFs and relieve intramolecular interactions. At the present time, four FH proteins are known to interact with Rho GTPases. DRF1 and DRF2 bind to RhoA, RhoB and RhoC (2,3). DRF2 also interacts with Cdc42Hs, but neither DRF1 nor DRF2 associate with Rac1 (2,3,6). The *S. cerevisiae* DRF, Bni1p, associates with Rho1p and Cdc42p (13,23). DRFs preferentially interact with GTP-bound Rho proteins and are inhibited by Rho inactivation (2,23). The product of formin related gene in leukocytes (*FRL*) was recently shown to interact with Rac1, but not with RhoA or Cdc42Hs (15). Thus, FH proteins appear to interact with specific Rho family GTPases.

One effect of Rho family GTPase and DRF activation is the induction of gene transcription from the SRE (5,6). The SRE is a short gene-regulatory sequence that is sufficient to activate transcription of immediate-early genes, such as *c-fos* and *β-actin*, in response to growth factor stimulation (24). Maximal activation of the *c-fos* SRE requires the formation of a complex between the ubiquitous transcription factor, SRF (25), and certain Ets-domain proteins, such as Elk1 and SAP1, which are collectively referred to as ternary complex factors (TCF). TCFs potentiate SRE transcriptional activation when they are phosphorylated by ERKs in response to Ras/Raf or Rac1/Cdc42/PAK signals (21,26-28). SRF activity is controlled independently of TCF activation, and is induced by activated forms of RhoA, Rac1, and Cdc42 (29). RhoA-induced activation is essential for SRE-serum responsiveness and requires the FH proteins, DRF1 (mDia1) and DRF2 (mDia2) (5,6,9). Rho activation however is not required for
Rac1 or Cdc42-induced SRF activation (29). Thus, multiple pathways target the SRF and TCF complex at the c-fos SRE and distinct Rac1 signals affect both subunits of the complex.

FHOS is a member of the formin homology (FH) family of proteins that is expressed at high levels in human splenic cells (30). FHOS contains all the conserved domains, including FH1, FH2, and FH3 domains, and a coiled-coil, that are characteristic of FH proteins. In this report, the identification of a C-terminal autoregulatory domain and a GTPase binding domain are described. The role of FHOS in Rho GTPase signaling pathways is also examined. In contrast to DRFs (mDia1 and mDia2) which are down-stream effectors of GTP-bound RhoA, FHOS binds to Rac1 in a guanine nucleotide-independent manner. Deletion of either the N- or C-termini created active forms of FHOS that stimulated transcription from the SRE. The N-terminal deletion mutant was localized to membrane ruffles and its ability to activate the SRE was blocked by overexpression of several Rac1 proteins. These data identify novel functions for FH proteins and Rac1 in the cell.
EXPERIMENTAL PROCEDURES.

**Plasmids**—The \( \text{pCMV5-HA-FHOS} \) full-length and \( \text{HA-FHOS(1-1010)} \) expression plasmids were previously described (30). For *in vitro* transcription reactions, these FHOS sequences were excised from \( \text{pCMV5} \) with \( \text{Asp718} \) and \( \text{BamHI} \) or \( \text{Asp718} \) and \( \text{SmaI} \), respectively, and subcloned into \( \text{pKS-Bluescript (pKS)} \). \( \text{pCMV5-HA-} \) and \( \text{pKS-FHOS (1-421)} \) were constructed by subcloning the \( \text{Asp718/HindIII} \) fragment from \( \text{pCMV5-HA-FHOS} \) into the base vectors. Similarly, \( \text{pKS-FHOS (1-321)} \) is the \( \text{Asp718/SalI} \) fragment from \( \text{pCMV5-HA-FHOS} \).

\( \text{pKS-FHOS (1-717)} \) was amplified with \( \text{Pfu Polymerase (Stratagene)} \) from \( \text{pCMV5-HA-FHOS} \) with gene-specific oligonucleotides (Integrated DNA Technologies, Inc) containing \( \text{Asp718} \) or \( \text{BamHI} \) restriction sites (GATGGTACCATGGCGGGGAAGA and TACGGATCCGGTGGCAGTGTGGT AGGCCGATGTTGATG). FHOS(469-1165) was constructed by amplifying a 248 base pair region of \( \text{pKS-FHOS} \) between nucleotides 1408 and 1648 with oligonucleotides containing \( \text{Asp718} \) or \( \text{BglII} \) restriction sites (AAGGTACCATGCCCAATGAGGCGG and CTAGATCTGAAAAGTCCAGGTCC), respectively. The resulting PCR product was subcloned into \( \text{pKS-FHOS} \) in place of the wild-type 5’-sequence. The fidelity of the \( \text{Pfu} \) polymerase and subcloning reactions were verified by automated DNA sequencing. FHOS (1-717) and (469-1165) were subcloned from \( \text{pKS} \) into the \( \text{pCMV5-HA-vector (30)} \) with \( \text{Asp718} \) and \( \text{BamHI} \).

For yeast-two-hybrid assays, \( \text{pAS-2-1-FHOS (1-328)} \) and (1-421) were generated by subcloning the \( \text{EcoRI/SalI} \) or \( \text{EcoRI/BamHI} \) fragments from the respective \( \text{pCMV5-HA-FHOS} \) vectors into \( \text{pAS2-1 (Clontech)} \). FHOS (491-1165) was created by subcloning the 2kB \( \text{PstI} \) fragment of \( \text{pCMV5-HA-FHOS (1-1165)} \) into \( \text{pAS2-1} \). \( \text{pAS2-1-FHOS (855-1165)} \) was...
made by removing the SalI fragment from \( pAS-2-1\)-FHOS(491-1165). To generate \( pAS2-1\)-FHOS (668-1165), the FHOS sequence was amplified by PCR with \( Pfu\) Turbo (Stratagene) and oligonucleotides, GTTGAATTCAACACCTCTTTTGCAGTC and GTTGGATCCTCAGGTTCCAGATAGAT (Integrated DNA Technologies). The purified PCR product was then subcloned into the EcoRI/BamHI sites of \( pAS2-1\).

Prokaryotic expression vectors for the GST-GTPase fusion proteins were kindly provided by Shuh Narumiya, \( pGEX-2T\)-RhoA, -Rac1, and Cdc42 (2); Gary Bokoch, \( pGEX-4T\)-Rac2 (31); Nora Heisterkamp, \( pGEX-2T\)-Rac3 (32); and Ulla Knaus, \( pGEX\)-Rac1->2 and -Rac 2->1 (33). \( pZipNeo\)-RacN17, Cdc42N17, and RhoAN19 expression vectors were obtained from Yi Zheng (34). Rob Lewis provided \( CMV\)-Rac1-myc and \( pCGT\)-RacV12 and -RacN17 (35).

**FHOS-GTPase Interaction Assays** - GST-GTPase fusion proteins were produced in *E.coli*, DH5\( \alpha\), during a three hour induction with 0.1mM IPTG. Bacteria were lysed by resuspension in Buffer A (10mM MES, pH6.5, 150mM NaCl, 2mM MgCl\(_2\), 0.5mM EDTA, 0.5% Triton X-100, 5mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride, 5\( \mu\)g/ml leupeptin, aprotinin, and pepstatin A) (2) and sonication. Insoluble material was separated by centrifugation. GST fusion proteins were purified from the lysates with glutathione-sepharose beads (Pharmacia). After washing the beads three times in Buffer A, fusion protein concentrations were estimated by comparison to BSA standards in Coomassie-stained SDS-PAGE gels. For reactions in the presence of GDP or GTP\(_\gamma\)S (Sigma), 10\( \mu\)M GST-GTPases on sepharose beads were preloaded overnight with 1mM guanine nucleotide (2). \(^{35}\)S-labeled FHOS proteins were *in vitro* transcribed and translated with...
the TNT T3 or T7-Coupled Reticulocyte Lysate Systems (Promega). One-tenth of the lysate was incubated with 400pmol of each nucleotide-loaded or unloaded GST-GTPase-beads for 90 minutes at 4°C. Beads were washed three times in Buffer A containing the appropriate guanine nucleotide. Proteins were resolved by 7% SDS-PAGE. The lower portion of each gel was stained with Coomassie dye to verify equal loading of the GST-GTPases to the reactions (data not shown). The upper portions of the gels were fixed in 40% methanol and 10% acetic acid, incubated with Amplify (Amersham Pharmacia Biotech), dried, and exposed to film.

For immunoprecipitation assays, two-10 cm plates of confluent MDA-231 cells were lysed on ice for 10 min in 1ml modified RIPA buffer (50mM Tris-HCl, pH7.5, 150mM NaCl, 1mM EDTA, 1% NP40, 0.25% sodium deoxycholate, 3µg/ml aprotinin, 1µM PMSF). Lysates were precleared with 50µl 50% Protein G-Sepharose slurry for 30 min at 4°C. Following centrifugation for 10min at 4000rpm, the lysates were collected and incubated overnight with 4µg anti-Rac mAb (UBI) or a control IgG mAb (MOPC-21, Sigma). Immune complexes were collected with 50µl 50% Protein-G-sepharose slurry for 2hr at 4°C, washed three times with modified RIPA buffer, resolved by 12% SDS-PAGE and transferred to Immobilon P (Millipore). Rac1 and FHOS were detected by immunoblotting with murine Rac1 (1:1000, UBI) and FHOS (1:600) (30) antibodies diluted in Tris buffered saline containing 0.1% Tween 20 and 5% non-fat dried milk. Proteins were visualized with SuperSignal West Pico Chemiluminescent Substrate (Pierce) and autoradiography.

For yeast-two-hybrid assays S.cerevisiae strain Y190 (MATa gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3, -112 +URA3::GAL -> lacZ, LYS2::GAL -> HIS3) were co-transformed with the indicated pAS-2-I-FHOS expression plasmid and pACT-FHOS (904-
Transformed yeast were selected on SD media lacking leucine (L), tryptophan (W) and histidine (H). Five individual colonies were restreaked onto SD media lacking HWL in the presence or absence of 50mM 2-aminotriazole.

Transfections and Reporter Assays-COS7 cells were seeded at a density of 5.2 cells/cm² in 12 well plates (2x10⁴ cells/well). Cells were allowed to adhere and grow for 4 to 16 hours in DMEM (Gibco BRL) containing L-glutamine, penicillin/streptomycin and 10% FBS (Biowhittaker). The pSRE, pAP-1, and NFκB luciferase (Luc) reporter plasmids contain three, four and four tandem copies of the respective promoter element fused to the Herpes simplex virus-thymidine kinase TATA-like promoter (Clontech). The SRE sequence is identical to the SRE in the c-fos promoter (24). Luciferase reporter plasmids (0.5µg) were mixed with 100ng pCMV5-secreted alkaline phosphatase (SEAP) and the indicated pCMV5-HA-FHOS or Rac expression vectors. Cells were transfected by incubation with reporter and expression plasmid mixtures in D-PBS (Biowhittaker) containing 0.5mg/ml DEAE-Dextran for 20 min at 37°C, followed by a 2.5 hr incubation in DMEM containing 5% FBS and 80µM chloroquine. After removal of the media, cells were shocked for 2.5 min with DMEM containing 10% DMSO and 5% FBS. The cells were then washed with D-PBS and incubated for 16 to 40 hr in DMEM containing 0.1% FBS.

Luciferase activity was measured with the Luciferase Assay Systems (Promega) as instructed by the manufacturer. SEAP activity was measured as previously described (36,37). Luciferase activity was normalized for transfection efficiency with the SEAP values for each sample. Fold activation was determined relative to samples transfected with pCMV5. Values
represent the mean of three independent transfections ± the standard error of the mean (SEM).

**Immunofluorescence.** NIH3T3 were grown on coverslips and transfected with the indicated FHOS expression plasmids with Superfect (Qiagen). C2C12 cells were transduced with the indicated MSCV-HA-FHOS vector using conventional methods (38). Forty hours later, cells were fixed with 2% paraformaldehyde, lysed in PBS containing 0.3% Triton X-100, and blocked for 30 min in IF buffer (3% BSA, 20mM MgCl₂, 0.3% Tween20, 1X PBS). Cells were incubated sequentially in IF buffer containing anti-HA mAb (clone 12CA5, Sigma), anti-mouse IgG-Alexa 546 (Molecular Probes), and anti-Rac1-FITC (UBI). Cells were mounted in 90% glycerol containing 0.4% N-propyl-gallate. Endogenous FHOS was detected in HeLa cells with FHOS antiserum (30). DNA was counterstained for 5 min with Hoescht’s dye (5µg/ml) as indicated.
**RESULTS**

*FHOS interacts with the Rho-family GTPase, Rac1.* FHOS is a recently described member of the Formin/Diaphonous family of proteins. Formin homology proteins interact with and are downstream effectors of Rho-family GTPases (2,3,6,15). To determine its molecular function, FHOS was tested for its ability to associate with the most frequently studied members of the Rho subfamily of GTPases, Cdc42Hs, Rac1 and RhoA. FHOS interacted with Rac1, but not with Cdc42Hs or RhoA (Figure 1A). The interactions between FHOS and Rac1 were detected in the presence of either GDP or GTP-γS (Figures 1A and 1B) and the absence of guanine nucleotides (Figure 1B). FHOS did not associate with Rac2 or Rac3, which are the Rho family GTPases that are most identical to Rac1, in any condition (Figure 1B). To verify the interaction between FHOS and Rac1 *in vivo*, lysates from human breast carcinoma cell line, MDA-231, were immunoprecipitated with Rac1 mAb. FHOS was detected in the Rac1 immune complexes by immunoblotting but not in control immunoprecipitates (Figure 1C). Moreover, Rac1 colocalized with FHOS in the cytoplasm of HeLa cells (Figure 1D). Thus FHOS interacts specifically with the Rho GTPase, Rac1, in an apparently guanine nucleotide-independent manner.

*FHOS interacts with the carboxy-terminus of Rac1.* The interaction between FHOS and Rac1, but not with Rac2 or Rac3 was somewhat surprising given the extensive sequence similarity between the three proteins (31,32). The extreme C-termini of Rac proteins however are poorly conserved (Figure 2A), and thus it was hypothesized that the C-terminus of Rac1 may mediate the interaction with FHOS. FHOS was tested for interactions with Rac1 and Rac2 chimeras in which the polybasic regions (amino acids 183-188) of each protein are interchanged (Figure 2A...
and B) (33). FHOS interacted with GST-Rac1 and the GST-Rac2 chimera containing the Rac1 polybasic domain (Rac2->1), but did not interact with GST, GST-Rac2, or the GST-Rac1 chimera containing six amino acids from Rac2 (Rac1->2) (Figure 2C). These data indicate that the polybasic domain in the Rac1 C-terminus mediates interactions with FHOS.

*Rac1 interacts with sequences in or near the FHOS FH1 domain.* To determine the region of FHOS that interacted with Rac1, truncated FHOS proteins (Figure 3A) were incubated with GST or GST-Rac1 in the absence of guanine nucleotides. Rac1 interacted with full-length FHOS (1-1165) as well as with FHOS proteins ((1-717) and (469-1165)) that retained the FH1 domain (Figure 3B). FHOS (1-421), which lacks the FH1 domain but contains the FH3 domain, did not interact with Rac1. Thus the Rac1 interaction domain of FHOS lies between the FH3 domain and the end of the FH1 domain (amino acids 422 to 717) (Figure 4C).

*FHOS Forms Intramolecular Interactions.* Rho family GTPases activate DRFs by disrupting intramolecular interactions between C- and N-terminal sequences in DRF proteins (3). To determine if FHOS forms intramolecular interactions, a C-terminal region of FHOS (residues 904-1165; Fc) (Figure 3A) was tested for interactions with various FHOS proteins. Full-length FHOS (1-1165) interacted strongly with GST-FHOS (904-1165) fusion protein, but not with the GST control (Figure 4B). To identify the region of FHOS that interacted with the C-terminus, several deletion mutants (Figure 3A) were generated and tested *in vitro* (Figure 4A) and in a yeast-two-hybrid binding assay (Figure 4B). FHOS proteins lacking the C-terminus (1-1010, 1-717, 1-490, 1-421, and 1-328) interacted with FHOS (904-1165). The N-terminal
truncation mutant, (469-1165), which lacks the FH3 domain but contains the FH1 and FH2 domains also associated with the C-terminus. The N-terminal truncation mutants, (491-1165), (668-1165) and (884-1165), however, did not interact with the C-terminus. These data suggest that residues between amino acids 1 to 490 and 904 to 1165 mediate intramolecular interactions in FHOS. The locations of these interaction domains relative to the Rac1 binding site are illustrated in Figure 4C.

FHOS stimulates transcription from the SRE. The interaction between FHOS and Rac1 is not dependent on GTP and suggests that FHOS is not an effector of Rac1. The unregulated nature of the FHOS-Rac1 interaction however does not exclude the possibility that FHOS is a component of a Rac1 signaling pathway or vice-versa. Activated Rac1 is a potent inducer of many signaling pathways, including SRE and ERK (29), JNK (39-41), and NFκB (42) pathways. The SRE is also a target of Rho-activated or truncated DRF proteins (2,3,5,6). FHOS proteins were therefore tested for their ability to activate a variety of Rac1-inducible reporters. As shown in Figure 5, FHOS proteins only activated the SRE-reporter. Although, full length FHOS (1-1165) did not effect on the basal activity of this reporter, N- and C-terminally-truncated FHOS proteins activated the SRE (Figure 5A). The C-terminal deletion mutant (1-1010), which retains the FH1 and FH2 domains, stimulated the SRE by three to eight-fold in low serum conditions (0.1% FBS) and in the absence of serum (data not shown). A C-terminal deletion mutant (1-717) that lacks the FH2 however did not activate transcription from the SRE. These results are consistent with the FHOS C-terminus being autoinhibitory and also indicated that the FH2 domain is required for SRE induction. The N-terminal FHOS truncation mutant (469-
1165) also stimulated transcription from the SRE even though it also interacted with the FHOS C-terminus (Figure 3B). It is speculated that the deletion weakened the intramolecular and created an active FHOS protein. Thus, removal of the N- and C-termini generates active FHOS proteins that stimulate transcription from the SRE.

The effects of the active FHOS proteins (1-1010) and (469-1165) were also tested on other Rac1-responsive pathways. In contrast to their effects on the SRE reporter, neither "activated" form of FHOS affected either the pAP-1 or NFκB reporters (Figure 5B and C). FHOS (1-1010) also had no effect on several other Rac1 inducible pathways including the E2F (43), Rb (43), and STAT3 (44,45) pathways (data not shown). Thus, FHOS activation appears to specifically effect signal transduction pathways that activate the SRE.

Rac1 Overexpression Represses FHOS-Induced SRE Activation. DRFs are downstream effectors of Rho GTPases (2,3,6). The guanine nucleotide-independent interaction between FHOS and Rac1 (Figure 1) suggested that the cellular function of FHOS and Rac1 interactions differs from that of DRF-Rho interactions. One possibility is that FHOS lies upstream of Rac1. Thus, the effects of dominant-negative Rac1 (N17) on SRE induction by FHOS proteins were examined. In these experiments, the N- and C-terminal FHOS truncation mutants each activated the SRE-reporter approximately three-fold, but the wild-type protein (1-1165) was without effect (data not shown). Rac N17 minimally repressed the basal activity of the SRE reporter by 1.7 fold (Figure 6A). The SRE-activity in cells transfected with full-length FHOS (1-1165) or C-terminal FHOS deletion mutant (1-1010) was similarly effected by Rac-N17 co-expression. In contrast, SRE activation by the N-terminal FHOS mutant (469-1165) was
repressed nearly three-fold by Rac N17. To determine the specificity of this result, dominant negative mutants of RhoA (N19) and Cdc42 (N17) were tested for their ability to repress FHOS-induced SRE transactivation. FHOS (469-1165) activity was not significantly altered by either Cdc42-N17 or RhoA-N19, but was once again inhibited by Rac-N17 (Figure 6B). In contrast, SRE activation by the C-terminal FHOS mutant (1-1010) was not affected by any of the dominant negative GTPases (Figure 6C). These data suggest that when activated, FHOS may effect both Rac1-dependent and –independent signaling pathways that terminate at the SRE.

If FHOS lies upstream of Rac1, then it would be expected that while overexpression of the dominant-negative Rac1 protein (N17) would repress FHOS-induced SRE activation, a constitutively-active Rac1 (V12) would not. Thus, several Rac1 proteins were tested for their effects on SRE activation by the N-terminal FHOS deletion mutant. FHOS (469-1165) activated the SRE six-fold in this experiment (Figure 6D). As previously shown, dominant negative Rac1 N17 repressed the basal activity of the promoter by approximately 40% (1.7 fold repression) and FHOS (469-1165) -induced activation by 56% (2.3 fold repression). Overexpression of wild-type Rac1 (myc-tagged) had similar effects on the basal and FHOS (469-1165)-induced activation. Consistent with a previous report (29), constitutively active Rac1 (V12) activated the basal activity of the promoter approximately two-fold. RacV12 however repressed the FHOS (469-1165)-induced activation by 51%. The ability of Rac1V12 to repress FHOS-induced activation to a similar extent as Rac1N17 and Rac-myc indicates that FHOS is not an upstream regulator of Rac1. Instead, it suggests that Rac1 may regulate FHOS signaling pathways.
**FHOS co-localizes with Rac1.** To begin to understand the mechanism by which Rac1 preferentially represses the N-terminal FHOS mutant (469-1165), the localization of these FHOS proteins was determined. Cellular fractionation experiments indicated that FHOS is predominantly cytoplasmic (30). Immunofluorescence imaging demonstrated that full-length FHOS (1-1165) is concentrated in the perinuclear region, although light staining is also observed in cellular extensions and membrane edges (Figures 1D and 7A). This staining pattern was observed for both endogenous (Figure 1D) and ectopic FHOS (Figure 7A) in multiple cell lines, does not appear to effect Rac1 localization (Figure 7B), and resembles that of mDia1 (2). The N-terminal mutant (469-1165) is concentrated in the perinuclear region like the wild-type protein and is detected at the cellular edges and membrane ruffles (Figure 7A). In contrast, the C-terminal truncation mutants, FHOS (1-1010) and (1-421), are diffusely localized throughout the cell (Figure 7A). Thus, the C-terminus may regulate the cellular localization of FHOS.
DISCUSSION

FHOS was previously characterized as a cytoplasmic protein with significant similarity to Formin and Diaphanous proteins (30). RNA blot analysis revealed that FHOS mRNA levels are very high in the spleen, skeletal muscle, and lung (30) (JJW, unpublished data). In this report, the first insights into the cellular functions and molecular regulation of FHOS are described. The data reported here demonstrate that FHOS may be regulated in a similar manner as other FH proteins but also identify novel mechanisms of regulation. The similarities between FHOS and other FH proteins include autoregulation by associations between N- and C-terminal residues, a specific interaction with a Rho family GTPase, and the ability of truncated forms to stimulate SRE-mediated transcriptional activation. Unlike what has been described for DRFs, FHOS associated with Rac1 in a guanine-nucleotide-independent manner in vitro. Overexpression of either dominant-negative or constitutively-active Rac1 proteins blocked SRE induction by an N-terminal FHOS truncation mutant but not by a C-terminal deletion mutant. These data suggest that FHOS may activate immediate early gene transcription from the SRE by multiple mechanisms, and that Rac1 may be a negative regulator of at least one FHOS signaling pathway.

FHOS is the second FH protein family member identified that interacts with Rac1. FRL also interacts with Rac1 but not with RhoA or Cdc42Hs (15). Rac1 interacted with N-terminal regions FHOS and FRL in the presence of either GDP or GTP (Figure 2) and (15). This is unlike the GTP-dependent Rho interactions with DRFs (2,3,6). The guanine-nucleotide-independent binding of Rac1 to FHOS and the inhibition of FHOS (469-1165)-induced SRE activation by active and inactive Rac1 proteins suggest that Rac1 binding may inhibit FHOS signaling pathways. Alternatively, FHOS may affect Rac1 activity. Because FHOS does not contain any
conserved domains found in GTPase-activating proteins (GAPs) or guanine nucleotide exchange factors (GEFs), it is unlikely that FHOS directly effects Rac1 activation. Moreover, inhibition of FHOS activity by both dominant-negative and constitutively active Rac1 proteins indicate that FHOS does not lie upstream of Rac1. One possibility is that FHOS acts as a scaffold to link Rac1 to other signaling molecules that associate with the FH1, FH2 and C-terminal domains. FHOS activation could thus affect the course Rac1 signaling pathways and the activation of effectors. The polybasic residues in the Rac1 C-terminus that are necessary for FHOS interaction are also required for maximum PAK1 activation and Rac1 homodimerization (33,46). Rac1 homodimerization potentiates the activity of PAK1 and other effectors (46). In preliminary studies, FHOS does not appear to associate with PAK1 (unpublished data). Thus by interacting with FHOS, Rac1 may activate PAK1-independent pathways (47). Additional studies are necessary to determine if any Rac1 effector molecules associate with FHOS and to identify the factors that regulate Rac1 binding to FHOS. It will also be important to determine if FHOS effects Rac1-dependent cytoskeletal organization and to identify the factors or signals that activate FHOS \textit{in vivo}.

The C-termini of FHOS and other FH proteins contain several important signaling and autoregulatory domains. Alberts recently described a Dia-autoregulatory domain (DAD) in the C-termini of DRF subfamily of FH proteins that interact with N-terminal sequences of the same molecule (9). FHOS shares limited sequence similarity with Dia proteins within the FH2 domain (30), but it does not appear to be a member of the DRF subfamily based on comparative sequence alignments of the full-length proteins (data not shown). A specific search for a DAD however identified twenty-six residues in the C-terminus of FHOS that aligns with 46% identity
to DADs in human DRF proteins (data not shown). The major difference in these DADs is a seven to nine amino acid spacer region in FHOS that separates the polybasic residues from the remainder of the motif. The DAD of the putative FH protein, KIAA1695, also contains a non-conserved spacer. Interestingly, Alberts’ data indicate that the DAD domain may have an effector function in addition to an autoinhibitory function (9). Biochemically, this model would seem intuitive, as releasing the DAD domain from intramolecular interactions should expose a protein-protein interaction surface. Our data indicate that region may also target FHOS to specific sites in the cell and thereby stimulate a specific signaling pathway(s).

The importance of the C-terminus is highlighted by the fact that the only known penetrant genetic mutations in FH proteins alter their extreme C-termini (16-20). Moreover, a C-terminal deletion mutant of DRF1 (mDia1) altered cytoskeletal organization in a manner consistent with Rho inactivation (3). It was proposed that defective intramolecular binding and the exposure of the FH1 and FH2 domains caused this phenotype. Our data suggest that C-terminal mutations may also effect FH protein localization and that the mislocalization could alter SRE regulation. Although FHOS (1-1010) appeared to be localized differently than the wild-type or N-terminal truncation mutant, it was still capable of activating the SRE. Rac1 however did not effect the activity of FHOS (1-1010). Preliminary data indicate that MEK inhibitors specifically block this pathway. Thus, the mechanisms by which mutations in the C-termini of FH proteins alter cellular and developmental phenotypes may involve differential protein localization and usage of differentially regulated signaling pathways.
ACKNOWLEDGMENTS

I thank Dr Scott Hiebert for his support, Drs. Rob Lewis, Sree Koka, David Largaespada, Carol Lange and Jim McCarthy for valuable discussions, and Xiaodong Li for technical assistance. I am grateful to Drs Gary Bokush, Nora Heisterkamp, Ulla Knaus, Rob Lewis, Shuh Narumiya, Yi Zheng for sharing their Rac reagents. This work was supported by the University of Minnesota Cancer Center, the Ingram Cancer Center at Vanderbilt University, the Minnesota Medical Foundation, and the National Institutes of Health, grant F32-CA77167.
REFERENCES

1. Wasserman, S. (1998) *Trends Cell Biol* 8(3), 111-5

2. Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B. M., and Narumiya, S. (1997) *Embo J* 16(11), 3044-56

3. Watanabe, N., Kato, T., Fujita, A., Ishizaki, T., and Narumiya, S. (1999) *Nat Cell Biol* 1(3), 136-43

4. Ishizaki, T., Morishima, Y., Okamoto, M., Furuyashiki, T., Kato, T., and Narumiya, S. (2001) *Nat Cell Biol* 3(1), 8-14.

5. Sotiropoulos, A., Gineitis, D., Copeland, J., and Treisman, R. (1999) *Cell* 98(2), 159-69.

6. Tominaga, T., Sahai, E., Chardin, P., McCormick, F., Courtneidge, S. A., and Alberts, A. S. (2000) *Mol Cell* 5(1), 13-25

7. Petersen, J., Nielsen, O., Egel, R., and Hagan, I. M. (1998) *J Cell Biol* 141(5), 1217-28

8. Castrillon, D. H., and Wasserman, S. A. (1994) *Development* 120(12), 3367-77

9. Alberts, A. S. (2000) *J Biol Chem* 16, 16

10. Uetz, P., Fumagalli, S., James, D., and Zeller, R. (1996) *J Biol Chem* 271(52), 33525-30

11. Kamei, T., Tanaka, K., Hihara, T., Umikawa, M., Imamura, H., Kikyo, M., Ozaki, K., and Takai, Y. (1998) *J Biol Chem* 273(43), 28341-5

12. Fujiwara, T., Mamamoto, A., Kim, Y., and Takai, Y. (2000) *Biochem Biophys Res Commun* 271(3), 626-9

13. Evangelista, M., Blundell, K., Longtine, M. S., Chow, C. J., Adames, N., Pringle, J. R., Peter, M., and Boone, C. (1997) *Science* 276(5309), 118-22

14. Imamura, H., Tanaka, K., Hihara, T., Umikawa, M., Kamei, T., Takahashi, K., Sasaki, T.,
and Takai, Y. (1997) *Embo J* 16(10), 2745-55

15. Yayoshi-Yamamoto, S., Taniuchi, I., and Watanabe, T. (2000) *Mol Cell Biol* 20(18), 6872-81.

16. Woychik, R. P., Stewart, T. A., Davis, L. G., D'Eustachio, P., and Leder, P. (1985) *Nature* 318(6041), 36-40

17. Woychik, R. P., Maas, R. L., Zeller, R., Vogt, T. F., and Leder, P. (1990) *Nature* 346(6287), 850-3

18. Maas, R. L., Zeller, R., Woychik, R. P., Vogt, T. F., and Leder, P. (1990) *Nature* 346(6287), 853-5

19. Lynch, E. D., Lee, M. K., Morrow, J. E., Welsh, P. L., Leon, P. E., and King, M. C. (1997) *Science* 278(5341), 1315-8

20. Bione, S., Sala, C., Manzini, C., Arrigo, G., Zuffardi, O., Banfi, S., Borsani, G., Jonveaux, P., Philippe, C., Zuccotti, M., Ballabio, A., and Toniolo, D. (1998) *Am J Hum Genet* 62(3), 533-41

21. Bar-Sagi, D., and Hall, A. (2000) *Cell* 103(2), 227-38.

22. Scita, G., Tenca, P., Frittoli, E., Tocchetti, A., Innocenti, M., Giardina, G., and Di Fiore, P. P. (2000) *Embo J* 19(11), 2393-8.

23. Kohno, H., Tanaka, K., Mino, A., Umikawa, M., Imamura, H., Fujiwara, T., Fujita, Y., Hotta, K., Qadota, H., Watanabe, T., Ohya, Y., and Takai, Y. (1996) *Embo J* 15(22), 6060-8

24. Treisman, R. (1990) *Semin Cancer Biol* 1(1), 47-58.

25. Norman, C., Runswick, M., Pollock, R., and Treisman, R. (1988) *Cell* 55(6), 989-1003.
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26. Shaw, P. E., Schroeter, H., and Nordheim, A. (1989) *Cell* **56**(4), 563-72.
27. Marais, R., Wynne, J., and Treisman, R. (1993) *Cell* **73**(2), 381-93.
28. Frost, J. A., Steen, H., Shapiro, P., Lewis, T., Ahn, N., Shaw, P. E., and Cobb, M. H. (1997) *Embo J* **16**(21), 6426-38.
29. Hill, C. S., Wynne, J., and Treisman, R. (1995) *Cell* **81**(7), 1159-70.
30. Westendorf, J. J., Mernaugh, R., and Hiebert, S. W. (1999) *Gene* **232**(2), 173-82.
31. Knaus, U. G., Heyworth, P. G., Kinsella, B. T., Curnutte, J. T., and Bokoch, G. M. (1992) *J Biol Chem* **267**(33), 23575-82.
32. Haataja, L., Groffen, J., and Heisterkamp, N. (1997) *J Biol Chem* **272**(33), 20384-8.
33. Knaus, U. G., Wang, Y., Reilly, A. M., Warnock, D., and Jackson, J. H. (1998) *J Biol Chem* **273**(34), 21512-8.
34. Fischer, R. S., Zheng, Y., and Quinlan, M. P. (1998) *Cell Growth Differ* **9**(3), 209-21.
35. Joneson, T., McDonough, M., Bar-Sagi, D., and Van Aelst, L. (1996) *Science* **274**(5291), 1374-6.
36. Berger, J., Hauber, J., Hauber, R., Geiger, R., and Cullen, B. R. (1988) *Gene* **66**(1), 1-10.
37. Westendorf, J. J., Yamamoto, C. M., Lenny, N., Downing, J. R., Selsted, M. E., and Hiebert, S. W. (1998) *Mol Cell Biol* **18**(1), 322-33.
38. Norris, P. S., Jepsen, K., and Haas, M. (1998) *J Virol Methods* **75**(2), 161-7.
39. Minden, A., Lin, A., Claret, F. X., Abo, A., and Karin, M. (1995) *Cell* **81**(7), 1147-57.
40. Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995) *Cell* **81**(7), 1137-46.
41. Olson, M. F., Ashworth, A., and Hall, A. (1995) *Science* **269**(5228), 1270-2.
42. Perona, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R., and Lacal, J. C. (1997) *Genes Dev* **11**(4), 463-75.

43. Gjoerup, O., Lukas, J., Bartek, J., and Willumsen, B. M. (1998) *J Biol Chem* **273**(30), 18812-8.

44. Turkson, J., Bowman, T., Adnane, J., Zhang, Y., Djeu, J. Y., Sekharam, M., Frank, D. A., Holzman, L. B., Wu, J., Sebti, S., and Jove, R. (1999) *Mol Cell Biol* **19**(11), 7519-28.

45. Simon, A. R., Vikis, H. G., Stewart, S., Fanburg, B. L., Cochran, B. H., and Guan, K. L. (2000) *Science* **290**(5489), 144-7.

46. Zhang, B., Gao, Y., Moon, S. Y., Zhang, Y., and Zheng, Y. (2001) *J Biol Chem* **276**(12), 8958-67.

47. Westwick, J. K., Lambert, Q. T., Clark, G. J., Symons, M., Van Aelst, L., Pestell, R. G., and Der, C. J. (1997) *Mol Cell Biol* **17**(3), 1324-35.
**Figure Legends**

**Figure 1.** FHOS interacts with Rac1 in a guanine nucleotide-independent manner. *A.* FHOS interacts with Rac1 but not RhoA or Cdc42. *In vitro* transcribed and translated $^{35}$S-labeled FHOS (1-1165) was co-incubated with GST (G) or the indicated GST-Rho family GTPase fusion protein in the presence of either 20µM GDP or GTP-$\gamma$S. Associated proteins were collected by glutathione affinity chromatography, resolved by SDS-PAGE and visualized by autoradiography. Input lanes contain 20% of $^{35}$S-FHOS added to GST binding reactions. *B.* FHOS does not interact with Rac2 or Rac3. GST pulldown assays were performed as described above except one reaction in each set lacked guanine nucleotides. *C.* FHOS and Rac1 associate *in vivo.* MDA-231 lysates were immunoprecipitated with Rac1 or control IgG. The upper portion of the membrane was immunoblotted with murine FHOS Ab. The bottom portion of the membrane was immunoblotted with Rac1 mAb. The position of the immunoglobulin light (IgL) chain is indicated. *D.* FHOS colocalizes with Rac1. Endogenous FHOS and Rac1 localization was determined in HeLa cells by immunofluorescence with FHOS or Rac1-specific antiserum. Cells were counterstained with Hoescht’s dye.

**Figure 2.** The carboxy-terminus of Rac1 mediates interactions with FHOS. *A.* Alignment of the C-terminal regions of human Rac protein sequences beginning with residue 160 of each protein. Bolded residues are unique to the respective Rac. *B.* Schematics of Rac1 and Rac2 chimeras. Residues 183-188 (KKRKRK of Rac1 and RQQKRA of Rac2) are swapped. *C.* *In vitro* transcribed and translated $^{35}$S-labeled FHOS (1-1165) was co-incubated with GST (G) or the indicated GST-Rac fusion protein in the absence of guanine nucleotides. Associated

25
proteins were detected as described in Figure 1. Input lanes contain 20% of $^{35}$S-FHOS added to GST binding reactions. GST-Rac1 was tested in duplicate.

**Figure 3.** Rac1 binds to a central domain in FHOS.  
*A.* Schematic of FHOS deletion mutants used in these studies. Relative positions of the formin homology (FH) 1, 2, and 3 domains, coiled-coil (CC) and putative diaphanous-related autoregulatory domain (DAD) are depicted.  
*B.* FHOS deletion mutants were in vitro-transcribed and translated in the presence of $^{35}$S methionine and cysteine and then incubated with GST or GST-Rac1 in the absence of guanine nucleotides. Associated proteins were detected as described in Figure 2. Input lanes contain 20% of $^{35}$S-FHOS added to GST binding reactions.

**Figure 4.** FHOS forms intramolecular interactions.  
*A.* FHOS N- and C-termini interact in vitro. *In vitro* transcribed and translated $^{35}$S-labeled FHOS proteins were co-incubated with GST (G) or GST-FHOS (904-1165) (Fc) immobilized on glutathione-sepharose beads. Associated proteins were resolved by SDS-PAGE and visualized by autoradiography. Input is 20% of the amount added to GST binding reactions.  
*B.* FHOS N- and C-termini interact in a yeast two hybrid assay. Y190 *S. cerevisiae* expressing GALAD-FHOS (904-1165) and the indicated GALDBD-FHOS fusion protein (left) were grown in the presence (right) or absence (middle) of 50mM 3-aminotriazole for 4 days. The growth of these colonies is representative of at least four clones from each transduction.  
*C.* Schematic summary of the relative positions of auto- and Rac1 interaction domains identified in this report.
**Figure 5. FHOS activates transcription from the SRE.** A. COS7 cells were co-transfected with 0.5µg pSRE-Luc and 0.1µg pCMV-SEAP reporter plasmids and 0, 1µg, 2.5µg, or 5µg of the indicated pCMV5-HA-FHOS expression plasmids. Luciferase activity was measured 20 hours after transfection and corrected for transfection efficiency with pCMV-SEAP activity. Values represent the mean of three independent transfections. B and C. COS cells were transfected with 2.5µg of the indicated “active FHOS” expression plasmid or pCMV5 and a pAP-1 reporter (B), which measures AP1 and SAPK/JNK activity, or a NFκB reporter (C). Fold activation is determined relative to cells transfected with the control vector.

**Figure 6. Rac1 overexpression blocks FHOS (469-1165)-induced SRE activation.** A. COS7 cells were transfected with the pSRE-Luc and pCMV-SEAP reporter plasmids and 1µg of the indicated pCMV5-HA-FHOS expression vector in the presence or absence of 1µg dominant negative Rac1 (N17) expression vector. Cells were grown in 0.1% FCS for 20 hrs. Relative luciferase values were normalized with SEAP activity. Fold repression of SRE activity in the presence of Rac-N17 was determined relative to the SRE-activity in cells transfected with pCMV5 or the indicated FHOS expression vectors only. Values represent the mean of three independent transfections. B and C. COS7 cells were co-transfected with pSRE-Luc and pCMV-SEAP reporter plasmids and 1µg pCMV and pCMV5-HA-FHOS (469-1165) (B) or pCMV5-HA-FHOS (1-1010) (C) in the absence (open bars) or presence of 2µg dominant negative Cdc42Hs-N17 (striped bars), Rac1-N17 (black bars), or RhoA-N19 (shaded bars) expression vectors. Luciferase activity was measured 40 hours after transfection. Values
represent the mean of two to three independent transfections. Similar results were obtained from parallel cultures after 20 hours (data not shown).  

D. COS7 cells were co-transfected with pSRE-Luc and pCMV-SEAP reporter plasmids and 1µg pCMV or pCMV5-HA-FHOS (469-1165) in the absence (open bars) or presence of 2µg of expression plasmids for wild-type Rac1-myc (shaded bars), dominant negative Rac1-N17 (black bars) or constitutively active Rac1-V12 (striped bars).

**Figure 7. The C-terminus of FHOS directs cellular localization.**  

A. Cellular localization of full-length and truncated FHOS proteins. The localization of the indicated HA-FHOS fusion proteins in NIH3T3 (top) or C2C12 (bottom) cells was analyzed by immunofluorescence with HA-specific antiserum.  

B. FHOS overexpression does not effect endogenous Rac1 localization. NIH3T3 cells were transiently transfected with HA-FHOS (1-1165) and analyzed by immunofluorescence with HA (left panel) and Rac1 (right panel) -specific antiserum.
Figure 1

A.

| Input | GDP | GTP-γS | GDP | GTP-γS | GDP | GTP-γS | GDP | GTP-γS |
|-------|-----|--------|-----|--------|-----|--------|-----|--------|
| GST   | Cdc42Hs | Rac1 | RhoA |

B.

| Input | GDP | GTP-γS | GDP | GTP-γS | GDP | GTP-γS | GDP | GTP-γS |
|-------|-----|--------|-----|--------|-----|--------|-----|--------|
| GST | Rac1 | Rac2 | Rac3 | RhoA |

C.

IgG  Rac1

| 215-122- | <-FHOS |
| 35-29-21- | <-IgL |
| <-Rac1 |

D.

FHOS

Rac1

DNA

Merge
Figure 2

A.

Rac1  LTQRLKTVFDEAIRAVLCPVVKKKRKRKCLL
Rac2  LTQRLKTVFDEAIRAVLCPQPTQQKRACSLL
Rac3  LTQRLKTVFDEAIRAVLCPVVKKPGKKCTVF

B.

- Rac1
- Rac2
- Rac1->2
- Rac2->1

C.

Input  GST  Rac1  Rac1  Rac2  Rac1->2  Rac2->1

FHOS--->
Figure 3

A.

1-1165  FH3  FH1  FH2  C  DAD
1-1010  FH3  FH1  FH2  C
1-717   FH3  FH1
1-490   FH3
1-421   FH3
1-328
469-1165  FH1  FH2  C  DAD
491-1165  FH1  FH2  C  DAD
668-1165  FH2  C  DAD
854-1165  C  DAD
904-1165 (Fc)  C  DAD

B.

Input  GST  GST-Rac1

215 -
122 -
79 -
47 -

1-1165  1-717  1-421  469-1165
1-1165  1-717  1-421  469-1165
1-1165  1-717  1-421  469-1165
Figure 4

A.

| 1-1165 | 1-1010 | 1-717 | 1-421 | 1-1165 | 1-1010 | 1-717 | 1-421 |
|--------|--------|-------|-------|--------|--------|-------|-------|
|        |        |       |       |        |        |       |       |

Input  G  Fc  G  Fc  G  Fc  G  Fc

FHOS (469-1165)

B.

pAS2-1

668-1165  1-490
854-1165  1-421
491-1165  1-328

-HWL

-HWL +3-AT

C.

| 1 | 328 | 421 | 491 | 717 | 1010 | 1165 |
|---|-----|-----|-----|-----|------|------|
|   | FH3 |     | FH1 |     | FH2  | C    |

N-terminal interaction domain

C-terminal interaction domain

Rac1 Interaction domain
Figure 5

A.

SRE

Fold Activation ± SEM

|          | 0  | 1  | 1.1165 | 2.5 | 1  | 1.1010 | 2.5 | 1  | 1.717 | 5  | 1  | 2.5 | (μg) |
|----------|----|----|--------|-----|----|--------|-----|----|-------|-----|----|----|------|
|          |    |    |        |     |    |        |     |    |       |     |    |    |      |
|          |    |    |        |     |    |        |     |    |       |     |    |    |      |
|          |    |    |        |     |    |        |     |    |       |     |    |    |      |
|          |    |    |        |     |    |        |     |    |       |     |    |    |      |
|          |    |    |        |     |    |        |     |    |       |     |    |    |      |

B.

AP-1

Fold Activation ± SEM

|          | pCMV5 | (1-1010) | (469-1165) |
|----------|-------|----------|-------------|
|          | 1     | 1        | 1.1010      |
|          |       | 0        |             |

NF-κB

Fold Activation ± SEM

|          | pCMV5 | (1-1010) | (469-1165) |
|----------|-------|----------|-------------|
|          | 1     | 1        | 1.1010      |
|          |       | 0        |             |
Figure 6

A. Fold Repression of FHOS-Induced SRE-Luc by RacN17 ± SEM

B. C. D.

Fold Activation ± SEM

|          | Control | Cdc42 N17 | Rac1 N17 | RhoA N19 |
|----------|---------|-----------|----------|----------|
| pCMV5    |         |           |          |          |
| (469-1165) |        |           |          |          |

|          | Control | Rac1-myc | Rac1-N17 | Rac1-V12 |
|----------|---------|----------|----------|----------|
| pCMV5    |         |          |          |          |
| (469-1165) |        |          |          |          |
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Jennifer J. Westendorf

*J. Biol. Chem. published online October 4, 2001*

Access the most updated version of this article at doi: 10.1074/jbc.M105162200

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