Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 38, Issue of September 23, pp. 23387–23390, 1994

An Anti-Ras Function of Neurofibromatosis Type 2 Gene Product (NF2/Merlin)*

(Received for publication, May 25, 1994, and in revised form, July 13, 1994)

Anjali Tikoo‡, Mikael Varga‡, Vijaya Ramesh§, James Gusella§, and Hiroshi Maruta‡¶

From the ‡Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria 3050, Australia, and the §Molecular Neurogenetics Unit, Neuroscience Center, Massachusetts General Hospital East, Charlestown, Massachusetts 02129-9142

Previously, we have cloned a candidate for the 595-amino acid neurofibromatosis type 2 tumor suppressor called NF2 or Merlin, with striking sequence similarity in its N-terminal half to an F-actin-binding protein family called TERM, which includes talin, ezrin, radixin, and moesin (Trofatter, J. A., MacCollin, M. M., Rutter, J. L., Murrell, J. R., Duyao, M. P., Parry, D. M., Eldridge, R., Kley, N., Menon, A. G., Pulaski, K., Haase, V. H., Ambrose, C. M., Munro, D., Bove, C., Haines, J. L., Martuza, R. L., MacDonald, M. E., Seizinger, B. R., Short, M. P., Buckler, A. J., and Gusella, J. F. (1993) *Cell* 72, 791–800). In an attempt to determine whether NF2 serves as a tumor suppressor and if so whether its N-terminal half is involved in its anti-oncogenicity, both full-length NF2 and its N-terminal half (NF2-N, residues 9–359) have been expressed in v-Ha-Ras-transformed NIH/3T3 cells. Like neurofibromatosis type 1 (NF1) fragments (Nur-E-Kamal, M. S. A., Varga, M., and Maruta, H. (1993) *J. Biol. Chem.* 268, 22331–22337), full-length NF2 can reverse the Ras-induced malignant phenotype, *i.e.* anchorage-independent growth in a soft agar, and restore contact inhibition of cell growth, indicating that NF2 is indeed a tumor suppressor. Furthermore, NF2-N also suppresses the Ras-induced malignant phenotype, although it appears to be less effective than the full-length NF2. These observations indicate that the anti-Ras function of NF2 resides in part in its N-terminal half. Thus, NF2 appears to be a new member of the tumor suppressor family of actin-cytoskeleton-associated proteins, which includes vinculin, α-actinin, tropomyosin-1, gelsolin, and tensin.

Oncogenic mutants of Ha-Ras, Ki-Ras, or N-Ras are found in around 30% of total human carcinomas, notably in 50% and 90% of colon and pancreatic carcinomas, respectively (1). Oncogenic mutants of Ras are locked in the active GTP-bound form (2). Their intrinsic GTPase activity is low and no longer stimulated by GTPase-activating proteins (GAPs)[1] such as GAP1, which is 1044 amino acids, and NF1, which is 2818 amino acids (3, 4).

We have shown previously that neurofibromatosis type 1 (NF1) fragments of 338 and 91 amino acids (NF338 and NF91) can reverse the malignant phenotype, *i.e.* anchorage-independent growth in a soft agar, caused by Ras mutants such as v-Ha-Ras (5). This was the first demonstration that a target of the tumor suppressor NF1 is the Ras-GTP complex as these NF1 fragments bind the Ras-GTP complex directly (5). Dysfunction or deletion of NF1 develops schwannomas and other tumors (neurofibromatosis 1) in central nervous system of human (6). We and another group recently cloned a candidate for the neurofibromatosis 2 tumor suppressor gene located on human chromosome 22q12 (7, 8). This gene encodes a protein of 595 amino acids called NF2 or Merlin (7, 8). Point mutations or truncation of NF2 have also been found to be closely associated with development of schwannomas and other central nervous system tumors including multiple meningiomas (7–11). Interestingly, NF2 shares around 50% sequence identity in its N-terminal half (residues 1–358) with the corresponding domains of several members of an F-actin-binding protein family called TERM, which includes talin, ezrin, radixin, and moesin (7, 8).

Very recently, at least four distinct actin-cytoskeleton-associated proteins were shown to act as tumor suppressors (12–15); overexpression of vinculin and α-actinin can reverse SV40-induced malignant transformation (12, 13), whereas tropomyosin-1 and gelsolin can reverse malignant transformation caused by Ras mutants such as v-Ha-Ras and v-Ki-Ras (14, 15). Interestingly, expression of the genes encoding these four actin-binding proteins is strongly suppressed when normal cells are transformed by SV-40 virus or Ras oncogenes (14). It is now clear that constitutive expression of these genes in SV40- or Ras-transformed cells results in reversion of the malignant state (12–15). Although NF2 is expressed widely in various human tissues (7), it still remains to be clarified whether NF2 expression is down-regulated by Ras or other oncogenes.

In this paper we demonstrate for the first time that (i) overexpression of NF2 can reverse the v-Ha-Ras-induced anchorage-independent growth of NIH/3T3 cells in a soft agar, (ii) the anti-Ras function of NF2 resides in its N-terminal half, and (iii) the level of NF2 mRNA is not down-regulated by v-Ha-Ras.

EXPERIMENTAL PROCEDURES

Construction of Plasmids Expressing Full-length NF2 and Its N-terminal Half in Mammalian Cells—An *Eco*RI DNA fragment of 1.8 kilobases encoding full-length human NF2 (7, 8) was prepared from JJR-1 cDNA (7) by polymerase chain reaction (PCR), and subcloned into retroviral vector pMV7 (16), which also expresses a neomycin resistance selectable marker. The orientation of the insert was determined by *Hin*dIII digestion, as one *Hin*dIII site is located 100 base pairs downstream of the *Eco*RI insertion site (16) and the other around codon 497 of NF2 DNA (8). Another *Eco*RI DNA fragment of 1.1 kilobases encoding the N-terminal half of NF2 (NF2-N, residues 1–359) was also prepared by PCR, and subcloned into vector pMV7 in essentially the same manner as described for full-length NF2. The orientation of the NF2-N insert was determined by *Hin*dIII digestion, as a second *Hin*dIII site is located around codon 289 of NF2-N (8). The resultant two plasmids, called NF2/pMV7 and NF2-N/pMV7, respectively, were purified by CsCl density gradient centrifugation for transfection.

* This work was supported in part by grants from the National Health and Medical Research Council of Australia and the Anti-Cancer Council of Victoria (to H. M.) and National Institutes of Health Grant NS24279 (to V. R. and J. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed. Fax: 613-347-1938; Tel.: 613-347-3155.

[1] The abbreviations used are: GAP, GTPase-activating protein; PCR, polymerase chain reaction; RT, reverse transcriptase; ACAP, actin-cytoskeleton-associated protein; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase kinase.

Effect of NF2 and NF2-N Transfection on the Colony-forming Ability of v-Ha-Ras-transformed Cells in Soft Agar—v-Ha-Ras-transformed NIH/3T3 cells were transfected with either NF2/pMV7 or NF2-N/pMV7 as complexes with liposomes as described previously (17). A parallel transfection with vector pMV7 alone was also carried out to serve as a negative control. The resultant G418-resistant transfectants were cloned in the presence of 400 μg/ml G418 (a neomycin analogue) as described previously (5). The colony formation in a soft agar (anchorage-independent growth) of NF2/pMV7 and NF2-N/pMV7 clones, in comparison with pMV7 clones, was examined by incubating 1000 cells/plate at 37 °C for 3 weeks under standard culture conditions (5). The colonies were stained with crystal violet and counted.

Reverse Transcriptase (RT) PCR Analysis of NF2, NF2-N, and v-Ha-Ras Expression—Total RNAs from both the parental v-Ha-Ras transformants and NF2/NF2-N transfectants were isolated by the method of Chomczynski and Sacchi (18). 5 μg of each total RNA preparation was then used as a template to synthesize v-Ha-Ras and NF2/NF2-N cDNAs by avian myeloblastosis virus reverse transcriptase (Promega) and a Riboclone cDNA synthesis kit (Promega), using the corresponding antisense PCR primers including the last several codons (17). To compare the amount of these cDNAs between the Ras transformants and NF2/NF2-N transfectants, the same aliquots of all cDNA preparations were then used as PCR templates for *Taq* DNA polymerase to amplify exogenous (human) or endogenous (mouse) NF2, NF2-N, or v-Ha-Ras cDNA sequences using the corresponding pairs of sense and antisense primers under essentially the same conditions as those described previously (17, 19). Under these PCR conditions, the amount of PCR products is linearly proportional to the initial amount of cDNAs used as templates and is far below the saturation point. The titration curves were drawn based on incorporation of [α-^{32}P]dGTP into the PCR bands, using both cloned v-Ha-Ras and NF2 DNAs at various concentrations (0.1–10 pg) as the standard PCR templates (data not shown).

The human and mouse NF2 amino acid sequences are 98% identical (20, 21), therefore, some pairs of the sense/antisense PCR primers corresponding to the human NF2 base sequences are able to amplify the corresponding base sequences of mouse NF2 cDNA as well. For instance, sense primer S and antisense primer B, corresponding to codons 9–14 and codons 354–359 of human NF2 (8), respectively, can be used to amplify both mouse and human sequences, whereas antisense primers A and C, corresponding to codons 335/336–340 (AGCGAGGCGCTGCCGC) and codons 590–595 (8), respectively, can be used for amplifying only human, and not mouse, sequences. Thus, PCR primers C and A were used to quantitate the levels of exogenous human NF2 and NF2-N mRNAs, respectively, whereas PCR primer B was used to compare levels of endogenous mouse NF2 mRNAs between normal and Ras-transformed NIH/3T3 cells or compare levels of endogenous (mouse) NF2 mRNAs in parental Ras transformants and exogenous (human) NF2/NF2-N mRNAs in NF2/NF2-N transfectants.

RESULTS

Anti-Ras Action of NF2—Using a retroviral vector pMV7, we have overexpressed full-length NF2 in v-Ha-Ras-transformed NIH/3T3 cells under the control of long terminal repeats, and examined the effects of NF2 on their growth, in particular the anchorage-independent growth in a soft agar, which correlates with their malignancy. As shown in Fig. 1 (*top panel*), very few NF2-overexpressing cells (*N*) formed large colonies in a soft agar. Under the same conditions, parental transformed cells (*T*) formed many large colonies. Each of these large colonies contains more than 100 cells. Furthermore, although these NF2 transfectants still formed small colonies, the number of small colonies derived from NF2 transfectants was only about 10% of that derived from parental transformed cells (see Table I, experiment 1). A quantitative RT PCR analysis of both v-Ha-Ras and NF2 mRNAs levels (see Fig. 1, *bottom panel*) has confirmed that this dramatic reduction in both size and number of colonies was due to expression of NF2, and not due to any reduction or loss of v-Ha-Ras expression. Thus, these data clearly indicate that, like NF1 fragments (5), full-length NF2 can reverse a malignant phenotype caused by v-Ha-Ras.

NF2 Restores Contact Inhibition of Cell Growth—Normal fibroblasts such as NIH/3T3 cells in a liquid culture cease to grow when they form a confluent monolayer on a glass or plastic substratum. This phenomenon is called contact (or density-dependent) inhibition of cell growth (or division). Interestingly, when the cells are transformed by v-Ha-Ras or many other oncogenes, they lose this contact inhibition and continue to grow, piling up on one another after they have formed the confluent monolayer. Interestingly, NF2 transfectants derived from v-Ha-Ras-transformed cells are contact-inhibited (see Fig. 2). In a liquid culture, both the control v-Ha-Ras transformants and their NF2 transfectants grow at the almost same rate until they form a confluent monolayer. However, at confluence, NF2 transfectants cease to grow (as do normal NIH/3T3 fibroblasts), whereas control transformed cells continue to proliferate (Fig. 2).

The Anti-Ras Function of NF2 Resides in Part in Its N-terminal Half—NF2 shares a high sequence identity (around 50%), mainly in the N-terminal domain (NF2-N, residues 1–358), with several other members of an F-actin-binding protein family (TERM) including talin, ezrin, radixin, and moesin (7). The remaining domain of NF2 has no sequence homology to any domains of the TERM proteins. Thus, if its actin binding is sufficient for anti-Ras action of NF2, NF2-N is expected to

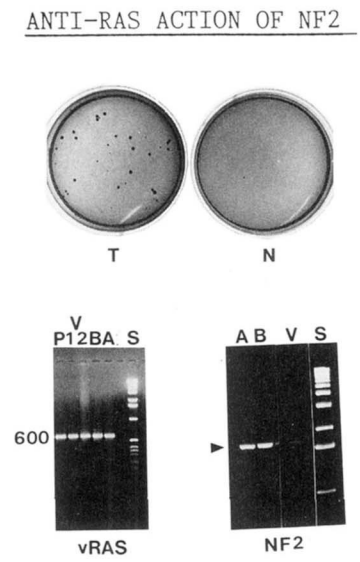

FIG. 1. *Top panel*, suppression of anchorage-independent growth of v-Ha-Ras transformants by NF2. v-Ha-Ras transformants (*T*), but not NF2 transfectants (*N*), grow rapidly in soft agar and form large colonies in an anchorage-independent manner. For details, see Table I. *Bottom panel*, expression of v-Ha-Ras and NF2 genes in NF2 transfectants. The steady-state levels of both v-Ha-Ras and human NF2 mRNAs in NF2-transfected clones (*A* and *B*), transfectants with vector alone (*V*, *1* and *2*), and parental v-Ha-Ras transformants (*P*) were estimated by a semi-quantitative RT PCR analysis using total RNAs extracted from each clone. *600*, v-Ha-Ras PCR product of 600 bp; *arrowhead*, NF2 PCR product of 1.8 kb; *S*, standard DNAs (1-kb ladder).

TABLE I
Soft agar colony-forming ability of NF2 and NF2-N transfectants derived from v-Ha-Ras transformants

The total number of colonies formed in soft agar per plate, 2 weeks after 1000 cells of each transfectant are plated, is shown. Each presented value was the average of the data from two independent experiments, and the standard deviation in each case was less than 5%.

Experiment 1 (NF2)		Experiment 2 (NF2-N)	
Vector pMV7 only	720 (100%)	Vector pMV7 only	826 (100%)
NF2 transfectants		NF2-N Transfectants	
Clone A	58 (8%)	Clone G	122 (15%)
Clone B	69 (10%)	Clone H	142 (18%)
Clone C	101 (14%)	Clone I	153 (19%)
Clone D	111 (16%)	Clone J	257 (32%)
Clone E	111 (16%)	Clone K	264 (33%)
Average	(13%)	Average	(23%)

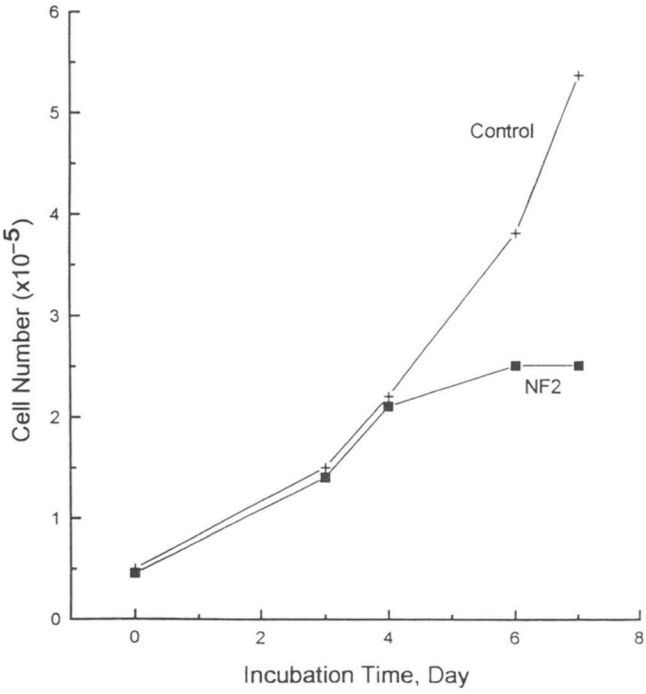

FIG. 2. **NF2 restores contact inhibition of cell growth that is lost in v-Ha-Ras transformants.** In a liquid culture, the v-Ha-Ras transformants (*Control*, *cross*) and their NF2 transfectants (*NF2*, *solid square*) grow at the almost same rate until they form a confluent monolayer (day 5–6). Then only NF2 transfectants cease to grow (as normal fibroblasts), whereas control transformants continue to grow and pile up on top of each other.

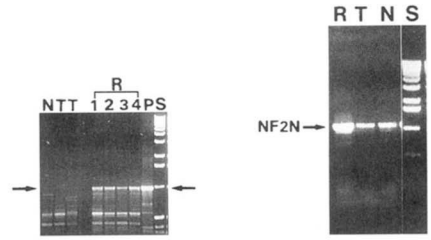

FIG. 3. *Left panel*, expression of NF2-N gene in NF2-N transfectants. The human NF2-N mRNAs could be detected only in NF2-N-transfected clones (*R*, *1–4*), but not in either v-Ha-Ras transformants (*T*) or normal fibroblasts (*N*), when primers A (human-specific) and S were used for semi-quantitative RT PCR analysis. The *arrow* indicates human NF2-N PCR product of 1 kb (codons 9–340). *P*, PCR product generated from a plasmid DNA carrying NF2-N; *S*, standard DNAs (1-kb ladder). *Right panel*, effect of v-Ha-Ras on expression of mouse NF2 gene. Using primers B (common to both human and mouse) and S, the steady-state levels of endogenous (mouse) NF2 mRNAs in normal (*N*) and v-Ha-Ras-transformed (*T*) cells were compared by semi-quantitative RT PCR analysis. No significant difference in NF2 mRNA level between normal and v-Ha-Ras-transformed cells could be detected. *NF2-N* indicates mouse/human NF2-N PCR products of 1.1 kb (codons 9–359). *R*, total human NF2-N/mouse NF2 level in NF2-N transfectants; *S*, standard DNAs (1-kb ladder).

suppress v-Ha-Ras-induced malignant transformation. As shown in Table I (experiment 2), NF2-N transfection indeed significantly reduces the colony-forming ability of v-Ha-Ras transformants in a soft agar, but its anti-Ras action (average 77% suppression) appears to be less efficient than that of full-length NF2 (average 87% suppression). Quantitative RT PCR analysis has revealed again that NF2-N transfectants express both NF2-N (Fig. 3) and v-Ha-Ras (data not known), clearly indicating that reduction of Ras-induced malignancy is due to NF2-N expression and not due to a loss of v-Ha-Ras expression. These observations confirmed our prediction that the anti-Ras function of NF2 resides in part in its highly conserved N-terminal half, which most likely binds actin filaments (F-actin).

NF2 mRNA Level Is Not Affected by v-Ha-Ras—Expression of the four distinct tumor suppressor genes encoding actin cytoskeleton-associated proteins (vinculin, α-actinin, gelsolin, and tropomyosin-1), which can suppress SV40- or Ras-induced malignancies, are down-regulated by either SV40 virus or oncogenic Ras mutants (14). Thus, we have compared the steady-state levels of NF2 mRNA between normal and v-Ha-Ras-transformed mouse NIH/3T3 cells by a quantitative RT PCR technique using a pair of oligonucleotide primers, B (antisense) and S (sense), that can amplify both mouse and human NF2 cDNAs (for detail, see "Experimental Procedures"). As shown in Fig. 3 (*right panel*), no significant difference in the NF2 message level could be detected between normal (*N*) and Ras-transformed (*T*) cells, and the endogenous (mouse) NF2 mRNA level in both cases is far lower than the exogenous (human) NF2-N mRNA level in NF2-N transfectants (*R*), clearly indicating that v-Ha-Ras does not affect the steady-state level of NF2 mRNAs.

Using another pair of primers, A (antisense) and S, which could amplify the exogenous (human) NF2 message of 1.0 kb in NF2-N transfectants (*R*, *1–R4*) but not the endogenous (mouse) NF2 cDNAs in either normal cells (*N*) or Ras transformants (*T*) (Fig. 3, *left panel*), we have confirmed that the 1.1-kb PCR products generated by primers B and S from both normal and Ras-transformed cells (Fig. 3, *right panel*) are indeed derived from mouse NF2 mRNAs, and not from a human NF2 cDNA contaminant (this conclusion was verified further by subsequent sequencing). The deduced human and mouse NF2 amino acid sequences are 98% identical (20, 21).

DISCUSSION

We have shown here that both full-length NF2 and its N-terminal half (NF2-N) can reverse the malignant phenotype induced by an oncogenic Ras mutant, but full anti-Ras action of NF2 seems to require its C-terminal half (NF2-C) as well. The partial requirement of NF2-C for full anti-oncogenicity appears to be compatible with previous observations that majority of mutations associated with neurofibromatosis 2 cause C-terminal truncations (7, 8), suggesting that the C-terminal domain is required for a potential tumor suppressor activity of NF2. In fact, our preliminary results indicate that an NF2-C fragment (residues 354–595) is anti-oncogenic as NF2-N.[2] Interestingly, one NF2 patient, represented by cell line GUS5722 (7), was found to carry an NF2 mutant that lacks 78 amino acids (residues 447–524) in the C-terminal domain (7). This missing domain contains two slightly overlapping Pro-rich decapeptide motifs (residues 478–495), which potentially could bind SH3 domains of some intracellular signal transducers such as the N-terminal domain of GAP1 (a Ras GTPase-activating protein of 120 kDa) and the C termini of myosin Is (single-headed myosin isoenzymes).

The domain NF2-N is highly homologous to several other members of the TERM family of F-actin-binding proteins (7, 8). In fact, our preliminary results indicate that NF2-N at least co-sediments with purified F-actin *in vitro*.[3] Thus, it appears that NF2 belongs to a tumor suppressor family of actin-cytoskeleton-associated proteins (ACAPs). This family includes at least four other ACAPs, *i.e.* vinculin, α-actinin, tropomyosin-1, and gelsolin (12–15). However, it still remains to be clarified whether their F-actin binding is absolutely required for their anti-oncogenicity. How do these actin-binding proteins block the oncogenic Ras signal transduction pathway? It was conceivable that expression of NF2 gene, like other members of this tumor suppressor gene family, could be suppressed by v-

[2] A. Tikoo and H. Maruta, unpublished observations.
[3] A. Tikoo and H. Maruta, unpublished observation.

Ha-Ras, and the constitutive expression of NF2 gene might be necessary to modulate the action of the Ras mutant. However, we have demonstrated here that v-Ha-Ras does not affect steady-state levels of NF2 mRNAs in NIH/3T3 cells. Alternatively, NF2 overexpression could lead to overproduction of actin or other ACAPs, which in turn suppress Ras-induced malignant transformation. Interestingly, it was recently reported that overexpression of the serum-responsive factor, which activates α-actin gene, can also suppress Ras-induced malignant transformation of NIH/3T3 cells (24). Thus, we are currently examining levels of actin and other F-actin-binding proteins in NF2 transfectants. It is equally possible that the NF2-F-actin complex might bind either Ras or one of the downstream targets of Ras (Raf, MAP kinase, MEK (MAP kinase kinase), or a target farther downstream), and therefore it could block the Ras-target interaction as small fragments of NF1 and N-terminal domain of Raf (5).[4] Thus, we are currently trying to find out which step of Ras signal transduction pathway (or kinase cascade) is blocked by NF2.

An interaction of Ras with F-actin itself or an actin-containing complex has been observed previously (25). Interestingly, it was demonstrated recently that a key role of Ras-GTP complex in Raf-induced activation of MEK is to recruit cytoplasmic Ser/Thr kinase Raf to the cytoskeleton (a detergent-insoluble fraction) located underneath the plasma membranes where activation of Raf takes place (26), but the nature of this cytoskeleton still remains to be determined. A rapid disruption of F-actin stress fibers (but neither microtubules nor intermediate filaments) is one of the early intracellular events that follow malignant transformation induced by SV40 virus, Ras, or other oncogenes (27). Overexpression of an N-terminal domain of GAP1 (GAP1N, residues 1–445), which contains a pair of SH2 domains and a single SH3 domain, also causes disruption of actin stress fibers (28). These observations indicate that GAP1N acts as a downstream target of Ras in this intracellular event and suggest the possibility that actin-cytoskeleton plays a key role in regulation of Ras-induced activation of Raf.

In this context, it is of interest to note that (i) another monomeric G protein (or GTPase) called Rho is required for organization of F-actin stress fibers (29), and (ii) p190, a GAP specific for Rho, directly interacts with GAP1N (22, 30, 31). GAP1N binds p190 only when p190 is phosphorylated at specific tyrosine residues (22). p190 activates Rho GTPase through its C-terminal domain (23, 31); however, it is possible that its N-terminal domain, which contains a G protein or GTPase motif, might act as a GTP-activated signal transducer as Ras and Rho (30). Perhaps p190 acts as a downstream target of Rho for organizing actin stress fibers and GAP1N somehow interferes with the action of p190 by forming a complex with tyrosine-phosphorylated p190. NF2 and other actin-binding tumor suppressors might serve as the downstream targets of p190 or form a complex with GAP1N, thereby interfering with GAP1N-p190 interaction to antagonize the oncogenic action of Ras mutants.

Acknowledgments—We are grateful to Masha Fridman for skillful assistance during preparation of mRNAs and Northern blot analysis, Giovanna D'Abaco for technical advice, and Dr. Tony Burgess for consistent encouragement and support, as well as invaluable advice and comment throughout this work. We also thank the JBC reviewers for their comments, which were helpful in improving the manuscript.

[4] M. Fridman, A. Tikoo, M. Varga, A. Murphy, and H. Maruta, submitted for publication.

REFERENCES

1. Bos, J. L. (1989) *Cancer Res.* **49,** 4682–4689
2. Polakis, P., and McCormick, F. (1993) *J. Biol. Chem.* **268,** 9157–9160
3. Vogel, U. S. A., Dixon, R. A., Schaber, M. D., Diehl, R. E., Marshall, E. M., Scolnick, E. M., Sigal, I. S., and Gibbs, J. B. (1988) *Nature* **335,** 90–93
4. Marchuk, D. A., Saulino, A. M., Tavakkol, R., Wallace, M. R., Andersen, L. B., Mitchell, A. L., Gutmann, D. H., Boguski, M., and Collins, F. (1991) *Genomics* **11,** 931–940
5. Nur-E-Kamal, M. S. A., Varga, M., and Maruta, H. (1993) *J. Biol. Chem.* **268,** 22331–22337
6. Xu, G., O'Connell, P., Viskochil, D., Cawthorn, R., Robertson, M., Culver, M., Dunn, D., Stevens, J., Gesteland, R., White, R., and Wiess, R. (1990) *Cell* **62,** 599–608
7. Trofatter, J. A., MacCollin, M. M., Rutter, J. L., Murrell, J. R., Duyao, M. P., Parry, D. M., Eldridge, R., Kley, N., Menon, A. G., Pulaski, K., Haase, V. H., Ambrose, C. M., Munro, D., Bove, C., Haines, J. L., Martuza, R. L., MacDonald, M. E., Seizinger, B. R., Short, M. P., Buckler, A. J., and Gusella, J. F. (1993) *Cell* **72,** 791–800
8. Rouleau, G. A., Merel, P., Lutchman, M., Sanson, M., Zucman, J., Marineau, C., Hoang-Xuan, K., Demczuk, S., Desmaze, C., Plougastel, B., Pulst, S. M., Lenoir, G., Bijlsmal, E., Fashold, R., Dumanski, J., De Jong, P., Parryl, D., Eldridge, R., Aurias, A., Delattre, O., and Thomas, G. (1993) *Nature* **363,** 515–521
9. Bianchi, A. B., Hara, T., Ramesh, V., Gao, J., Klein-Szanto, A. J. P., Morin, F., Menon, A. G., Trofatter, J. A., Gusella, J. F., Seizinger, B. R., and Kley, N. (1994) *Nature Genet.* **6,** 185–192
10. Twist, E. C., Ruttledge, M. H., Rousseau, M., Sanson, M., Rapi, L., Merel, P., Delattre, O., Thomas, G., and Rouleau, G. A. (1994) *Hum. Mol. Genet.* **3,** 147–151
11. Jacoby, L. B., MacCollin, M., Louis, D. N., Mohney, T., Rubio, M. P., Pulaski, K., Trofatter, J. A., Kley, N., Seizinger, B., Ramesh, V., and Gusella, J. F. (1994) *Hum. Mol. Genet.* **3,** 413–419
12. Fernandez, J. L. R., Geiger, B., Salomon, D., Sabanay, I., Zoeller, M., and Ben-Ze'ev, A. (1992) *J. Cell Biol.* **119,** 427–438
13. Glueck, U., Kwiatkowski, D., and Ben-Ze'ev, A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 383–387
14. Prasad, G. L., Fulder, R. A., and Cooper, H. L. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 7039–7043
15. Muellauer, L., Fujita, H., Ishizaki, A., and Kuzumaki, N. (1993) *Oncogene* **8,** 2531–2536
16. Kirschmeir, P. T., Housey, G. M., Johnson, M. D., Perkins, A. S., and Weinstein, I. B. (1988) *DNA* **7,** 219–225
17. Nur-E-Kamal, M. S. A., Sizeland, A., D'Abaco, G., and Maruta, H. (1992) *J. Biol. Chem.* **267,** 1415–1418
18. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159
19. Fath, I., Schweighoffer, F., Rey, I, Multon, M. C., Boiziau, J., Duchesne, M., and Tocque, B. (1994) *Science* **264,** 971–974
20. Hara, T., Bianchi, A. B., Seizinger, B. R., and Kley, N. (1994) *Cancer Res.* **54,** 330–335
21. Haase, V. H., Trofatter, J. A., MacCollin, M., Tarttelin, E., Gusella, J. F., and Ramesh, V. (1994) *Hum. Mol. Genet.* **3,** 407–411
22. Ellis, M., Moran, M., McCormick, F., and Pawson, T. (1990) *Nature* **343,** 377–381
23. Morii, N., Kumagai, N., Nur-E-Kamal, M. S. A., Narumiya, S., and Maruta, H. (1993) *J. Biol. Chem.* **268,** 27160–27163
24. Kim, J. H., Johansen, F. E., Robertson, N., Catino, J. J., Prywes, R., and Kumar, C. C. (1994) *J. Biol. Chem.* **269,** 13740–13743
25. Maruta, H. (1989) in *Ras Oncogenes* (Spandidos, D. A., ed) pp. 255–260, Plenum Publishing Co., New York
26. Stokoe, D., MacDonald, S. G., Cadwallader, K., Symons, M., and Hancock, J. F. (1994) *Science* **264,** 1463–1467
27. Weber, K., Lazarides, E., Goldman, R. D., Vogel, A., and Pollack, R. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39,** 363–369
28. McGlade, J., Brunkhorst, B., Andersen, D., Mbamalu, G., Settleman, J., Dedhar, S., Rozakis-Adcock, M.,, Chen, L. B., and Pawson, T. (1993) *EMBO J.* **12,** 3073–3081
29. Patterson, H. F., Self, A. J., Garret, M. D., Just, I., Aktories, K., and Hall, A. (1990) *J. Cell Biol.* **111,** 1001–1007
30. Settleman, J., Narashimhan, V., Foster, L. C., and Weinberg, R. A. (1992) *Cell* **69,** 539–549
31. Settleman, J., Albright, C. F., Foster, L. C., and Weinberg, R. A. (1992) *Nature* **359,** 153–154