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Identification and characterization of the neurofibromatosis type 1 protein product

JEFFREY E. DECLUE, BRUCE D. COHEN, AND DOUGLAS R. LOWY

Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20892

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ABSTRACT **The neurofibromatosis type 1 (NF1) gene responsible for von Recklinghausen neurofibromatosis is related to regulators of ras proteins, and a portion of NF1 that is homologous to the ras GTPase-activating protein (GAP) encodes a similar GTPase-stimulating activity. We have raised rabbit antisera to a bacterially synthesized 48-kDa peptide corresponding to the GAP-related domain of NF1 (NF1-GRD). These antisera immunoprecipitated the NF1-GRD peptide, and one of them specifically inhibited the GTPase-stimulating activity of NF1-GRD. The sera specifically detected a 280-kDa protein in lysates of mouse NIH 3T3 and human HeLa cells. This protein corresponds to the NF1 gene product, as shown by several criteria, including partial proteolysis. Subcellular fractionation revealed that while GAP is predominantly cytoplasmic, all of the NF1 was recovered in a pellet (100,000 × *g*) fraction. NF1 was present in a large molecular mass complex in fibroblast and Schwannoma cell lines and appears to associate with a very large (400–500 kDa) protein in both cell types. The relevance of these findings to cellular regulation of p21^{ras} is discussed.**

The *ras* genes (Ha-*ras*, Ki-*ras*, and N-*ras*) encode 21-kDa proteins (p21^{ras}) that are required in normal mitogenic signaling and are mutationally activated in many different types of tumors. The p21^{ras} proteins bind guanine nucleotides with high affinity and can hydrolyze GTP to GDP (1–4). They are biologically active when bound to GTP and inactive when bound to GDP (5–7).

In mammalian cells, p21^{ras} can be negatively regulated by the GTPase-accelerating protein (GAP), a 120-kDa cytoplasmic protein that is an enzymatic stimulator of the intrinsic GTPase of p21^{ras} (5, 8). p21^{ras} proteins that are mutationally activated are resistant to this enzymatic activity of GAP. The C-terminal one-third of GAP specifies the GTPase acceleration function, and this region of GAP is homologous to a segment of the yeast *IRA1* and *IRA2* genes, which encode negative regulators of RAS proteins in *Saccharomyces cerevisiae* (9, 10). When expressed in yeast cells deficient for *IRA* function, GAP can complement the loss of *IRA* (11, 12).

The region of chromosome 17 that carries the gene for the von Recklinghausen form of neurofibromatosis (NF1) has recently been identified, and part of the NF1 gene has been molecularly cloned (13, 14). The NF1 protein is apparently encoded by a very large (11–13 kilobases) mRNA that is expressed in many cell types. Analysis of the cloned portion of the human NF1 cDNA shows a very large open reading frame of >2485 codons whose 5′ end has not been identified (15). The predicted polypeptide encoded by the open reading frame has extensive homology with *IRA1* and *IRA2* and with the C-terminal portion of GAP that is homologous to the *IRA* genes. This GAP-related domain (GRD) encoded by *NF1* shares several properties with GAP. NF1-GRD can accelerate the intrinsic GTPase activity of normal p21^{ras}, mutationally activated p21^{ras} is resistant to this activity, and loss of IRA function in *S. cerevisiae* can be complemented by NF1-GRD (16–18).

With the long-term goals of gaining further insight into the regulation of mammalian p21^{ras} protein and the role of NF1 and ras proteins in the development of neurofibromatosis, we have raised antibodies against NF1-GRD protein expressed in bacteria. These antibodies specifically recognize a protein of 280 kDa in murine and human cells. We report here the identification of this 280-kDa protein as the *NF1* gene product and characterize some of its biochemical properties.

MATERIALS AND METHODS

Cloning and Bacterial Expression of NF1-GRD and Immunization. A fragment of NF1 cDNA in the plasmid clone pAcNF1-GRD (16) was provided by F. McCormick (Cetus). This plasmid was used as a template for PCR synthesis of a fragment with the oligomers JD17 (GGTCTAGAAAGGAGGTACGATCATGGAAGCCAAATCACAG) and JD18 (GGAGATCTTCATAACGTTTTCAAAGC) for 35 cycles as described (19). The resulting fragment encodes the same 474 amino acids of NF1 as described by Martin *et al.* (16). It was digested with *Xba* I and *Bgl* II (sites underlined) and cloned into Bluescript SK− (Stratagene) to yield the plasmid pJDC169 and into the plasmid pXCRAsn17 (20), which had been cleaved with *Xba* I and *Bam*HI to remove the *ras* gene insert, to yield the plasmid pJDC170. Plasmid pJDC169 was used for *in vitro* transcription/translation (see below), while pJDC170 was used to transform the *lacIq*$^{+}$ strain JM109 (Promega). NF1-GRD expression was induced with isopropyl β-D-thiogalactopyranoside (BRL), and the cells were lysed by sonication. The NF1-GRD, which was found in the insoluble fraction, was purified from polyacrylamide gels, concentrated by centrifugation in a Centricon cartridge (Amicon), and 100–200 μg was used to immunize New Zealand White rabbits. Booster injections were carried out 3 and 5 weeks later.

Immunoprecipitation and *in Vitro* Translation. Cells were labeled 20–22 hr at a density of 2 × 10^{6} cells per T-25 flask in cysteine- and methionine-free medium (ICN) with 2% fetal bovine serum and 250 μCi of Tran^{35}S-label and [^{35}S]cysteine (ICN) per ml (1 Ci = 37 GBq). Cells were rinsed once with ice-cold phosphate-buffered saline (PBS) and lysed in 400–1000 μl of lysis buffer [20 mM Tris·HCl, pH 7.4/100 mM NaCl/5 mM $MgCl_2$/1% Nonidet P-40/0.5% sodium deoxycholate/1 mM dithiothreitol (DTT)/16 μg of aprotinin per ml]. Lysates were clarified by microcentrifugation for 10 min at 10,000 × *g* at 4°C. Immunoprecipitation was carried out as follows: 300–500 μl of lysate was incubated with 5 μl of antiserum and 60 μl of a 10% suspension of protein A-Sepharose for 2 hr at 4°C. The immunoprecipitates were washed two times with wash buffer 1 (10 mM $NaPO_4$, pH 7.6/100 mM NaCl/1 mM EDTA/1% Triton-X 100/0.5% sodium deoxy-

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Abbreviations: GAP, GTPase-activating protein; GRD, GAP-related domain; DTT, dithiothreitol; NF1, neurofibromatosis type 1.

cholate/0.1% SDS), two times with wash buffer 2 (20 mM Tris·HCl, pH 8.3/250 mM NaCl/1% Nonidet P-40/0.1% SDS), then two more times with wash buffer 1, and boiled in SDS sample buffer. For immunoprecipitation of *in vitro* translation products, 7 μg of pJDC169 was first linearized with *Hin*dIII, precipitated, and used to program a 100-μl transcription reaction mixture with T3 polymerase according to specifications (Promega). The transcription reaction mixture was split 1:4 and precipitated in ethanol. One-fourth of the product was used to program a 50-μl translation reaction mixture with rabbit reticulocyte lysate (Promega), and 5 μCi of Tran^{35}S-label (ICN) for 60 min at 30°C. Five microliters of translation product was then added to 400 μl of lysis buffer and treated as described above. SDS/polyacrylamide gel electrophoresis of NF1-GRD was carried out on 12% gels; analysis of the 280-kDa NF1 protein was on 6% gels. Molecular size standards were from Pharmacia.

Cell Fractionation. One confluent T-175 flask of NIH 3T3 cells was labeled for 20 hr with ^{35}S-labeled amino acids as described above and rinsed with PBS; the cells were removed from the flask by scraping in 5 ml of cold PBS and pelleted. The cells were resuspended in 1 ml of hypotonic buffer (10 mM Tris·HCl, pH 8.0/1 mM DTT/16 μg of aprotinin per ml), left on ice 10 min, and then lysed by 50 strokes in a Dounce homogenizer (Wheaton). The nuclei and unlysed cells were removed by centrifugation at 1900 rpm for 5 min, and the supernatant was spun at 100,000 × *g* for 30 min at 4°C. The pellet from the 100,000 × *g* spin (P100) was resuspended in 2 ml of lysis buffer (see above), while the supernatant (S100) was brought up to a 2-ml volume and 1× lysis buffer concentration. Then 0.2 ml of each fraction was subjected to immunoprecipitation in antibody excess with anti-GAP antiserum A (21), anti-NF1 immune antiserum 31, or preimmune serum as described above.

Glycerol Gradient Sedimentation Analysis. Cells were plated at 5 × 10^6 cells per T-75 flask, and two or three flasks were labeled as described above and lysed in 250 μl of lysis buffer containing 4% (vol/vol) glycerol and lacking DTT. The lysates were loaded onto 5 ml of 9–14% continuous glycerol gradients, centrifuged in an SW50.1 rotor at 48,000 rpm for 4 hr, and 250- or 500-μl fractions were removed, divided in half, and subjected to immunoprecipitation with preimmune or anti-NF1 antiserum as described above. The immunoprecipitates were washed four times with the modified lysis buffer and analyzed on 6% polyacrylamide gels. To disrupt potential complexes either before or after spinning through glycerol gradients, SDS and 2-mercaptoethanol were added to concentrations of 0.5% and 0.1%, respectively, and the lysates were boiled for 3 min. Gel filtration calibration standards were from Sigma.

RESULTS

Development and Characterization of Anti-NF1-GRD Antiserum. To obtain antiserum that might recognize the NF1 protein, the NF1-GRD sequence was expressed in *Escherichia coli*, and the resulting 48-kDa NF1-GRD peptide was purified by gel electrophoresis and injected into two rabbits. Since the NF1-GRD peptide used as the immunogen can accelerate the intrinsic GTPase activity of normal p21^{ras}, the effect of the rabbit serum on this activity was examined. As previously reported (16), immunoaffinity-purified, baculovirus-derived NF1-GRD stimulated the hydrolysis of [γ-^{32}P]GTP that had been prebound to *E. coli*-produced p21^{ras} (Fig. 1, +NF1). One of the two immune antisera (no. 30) significantly inhibited the GTPase acceleration when this serum was preincubated with the NF1-GRD peptide (Fig. 1 *Left*). The second antiserum (no. 31) had little if any inhibitory activity toward the NF1-GRD peptide (Fig. 1 *Center*). The inhibitory effect of antiserum 30 was specific for the NF1-GRD peptide in that it did not inhibit the ability of GAP to accelerate the GTPase activity of the p21^{ras} (Fig. 1 *Right*).

As an additional test of the rabbit serum, *in vitro* translated ^{35}S-labeled NF1-GRD was incubated with the serum and subjected to immunoprecipitation. Immune antiserum from each rabbit immunoprecipitated similar amounts of *in vitro* translated 48-kDa NF1-GRD peptide (Fig. 2*A*, arrowhead). Neither of the rabbit preimmune sera immunoprecipitated the NF1-GRD peptide (Fig. 2*A*). We conclude that although both sera recognize epitopes in the NF1-GRD peptide, they differ in that one serum can effectively neutralize the enzymatic activity of this region, while the other does not appreciably inhibit the activity.

The NF1 Gene Product Is a 280-kDa Protein. To determine whether the sera would detect specific proteins in mammalian cells, mouse NIH 3T3 and human HeLa cells were metabolically labeled with ^{35}S-labeled amino acids, and cell extracts were immunoprecipitated with preimmune and immune serum 31 and analyzed by polyacrylamide gel electrophoresis (Fig. 2*B*). The large reported size of the NF1 mRNA and sequence analysis of the partial cDNA suggested that the full-length NF1 protein would be at least 250 kDa (14, 15). In accordance with this prediction, a 280-kDa band was detected in both cell types. The same band was also specifically recognized by immune antiserum 30, although somewhat less efficiently (data not shown). The conservation of the 280-kDa

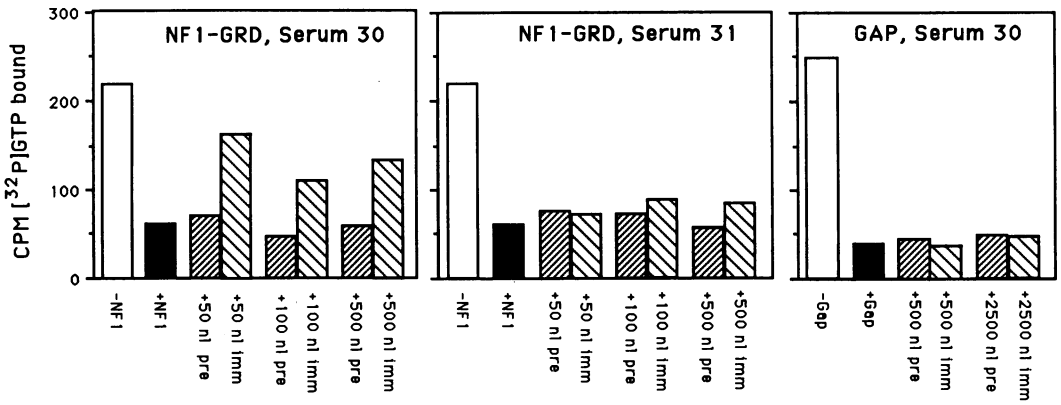

FIG. 1. Inhibition of GTPase-stimulating activity of NF1-GRD by immune serum. Affinity-purified GTPase stimulator (NF1-GRD or GAP) (16) was preincubated with the indicated amount of antiserum from rabbit 30 or 31 in reaction buffer for 60 min at 4°C. p21^{ras} was bound with [γ-^{32}P]GTP, and reactions were then carried out as described (16, 22) with 50 ng of p21^{ras} in a 50-μl vol. The [γ-^{32}P]GTP remaining bound to p21^{ras} was assayed by filter binding as described (22). Each determination represents the mean of duplicate data points. pre, Preimmune serum; imm, immune antiserum.

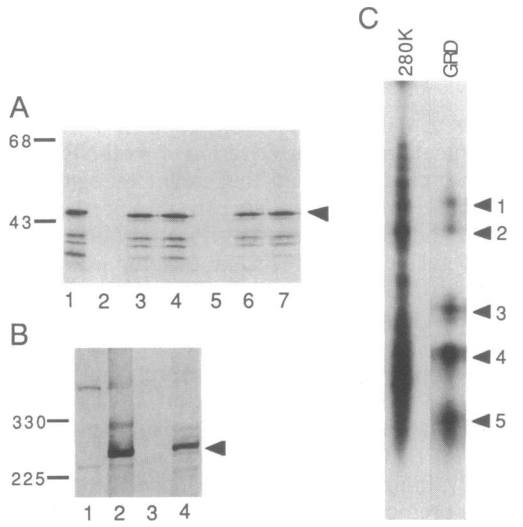

FIG. 2. Immunoprecipitation of NF1 proteins derived from *in vitro* translation of NF1-GRD (*A*) or labeled NIH 3T3 and HeLa cell lysates (*B*). (*C*) Comigrating cleavage products produced by cyanogen bromide treatment of 280-kDa band from NIH 3T3 and NF1-GRD. (*A*) Five microliters of *in vitro* translated NF1-GRD was either run directly on a 12% polyacrylamide gel (lane 1) or subjected to immunoprecipitation with 5 μl of preimmune serum (lanes 2 and 5), 0.5 μl of immune antiserum (lanes 3 and 6), or 1.0 μl of immune antiserum (lanes 4 and 7). Lanes 2–4, reactions with serum from rabbit 30; lanes 5–7, reactions with serum from rabbit 31. Arrowhead, migration of NF1-GRD; the faster-migrating bands represent products initiated at downstream methionine codons. (*B*) Labeled cultures of NIH 3T3 cells (lanes 1 and 2) or HeLa cells (lanes 3 and 4) were subjected to immunoprecipitation with 5 μl of preimmune (lanes 1 and 3) or immune (lanes 2 and 4) serum. Arrowhead, position of 280-kDa NF1 protein. (*C*) ^{35}S-labeled 280-kDa band isolated by immunoprecipitation and *in vitro* translated NF1-GRD were electrophoresed in glass cylinders using a discontinuous buffer SDS gel system of 5% or 10%, respectively. The first dimension cylinders were incubated with cyanogen bromide (40 mg/ml) at 45°C for 3 hr and then electrophoresed in a SDS/15% polyacrylamide gel.

protein from HeLa and NIH 3T3 cells was verified by analysis of proteolytic cleavage products (data not shown). This conservation is consistent with it representing the NF1 gene product, since RNA hybridization and sequence analysis have demonstrated considerable homology between NF1 sequences of murine and human origin (14, 23).

Further analysis also supported the conclusion that the 280-kDa band represents the full-length NF1 protein product. When immune antiserum 31 was incubated with the NF1-GRD peptide purified from *E. coli*, immunoprecipitation of the 280-kDa band from HeLa cells was partially blocked, while bovine serum albumin had no effect (data not shown). Furthermore, when [^{35}S]-labeled, *in vitro* translated NF1-GRD peptide and the 280-kDa band immunoprecipitated from NIH 3T3 cells were subjected to partial cleavage with cyanogen bromide, several comigrating cleavage products were produced (Fig. 2*C*). While the *in vitro* translated NF1-GRD yielded five major cleavage products (designated 1–5 in Fig. 2*C*), the 280-kDa band immunoprecipitated from NIH 3T3 cells generated at least three bands that comigrated with NF1-GRD cleavage products (bands 1, 2, and 4). It could not be determined whether a product from the 280-kDa band comigrated with cleavage product 5. Band 3 may represent a cleavage product bordering an end of the NF1-GRD (an end cleavage product of the NF1-GRD peptide would be expected to be part of a larger cleavage product in the full-length NF1 protein, since the full-length NF1 protein extends in both directions beyond the NF1-GRD peptide). Therefore, the comigration of three cleavage products is highly significant. In addition, a serum generated by D. Gutmann and F. Collins (University of Michigan Medical School), which was raised against a NF1 peptide ≈700 amino acids upstream from the GRD, specifically recognized a protein whose migration rate was identical to that of the 280-kDa protein described here (data not shown). Finally, we have found that a Schwannoma cell line derived from a NF1 patient fails to produce any detectable 280-kDa protein (J.E.D. and D.R.L., unpublished data). Thus, by a variety of criteria, the 280-kDa protein represents the authentic NF1 gene product.

Subcellular Localization of NF1 Protein. To determine the subcellular localization of the NF1 protein, labeled NIH 3T3 cells were lysed in the absence of detergent and separated into particulate (P100) and nonparticulate (S100) fractions. By immunoprecipitation analysis, GAP was found primarily in the soluble fraction (Fig. 3 *Left*), as reported previously (5). In contrast, we found that NF1 was reproducibly recovered entirely in the P100 fraction (Fig. 3 *Right*). This result raised the possibility that the NF1 protein might associate primarily with the plasma membrane or with intracellular organelles. Immunofluorescence analysis with serum 31 in NIH 3T3 cells yielded a staining pattern that was distinct from the plasma membrane staining of p21^{ras} (24) (data not shown). Further studies need to be directed at understanding the localization of NF1 to the particulate fraction.

Pulse–chase analysis of the 280-kDa NF1 protein in NIH 3T3 cells indicated that the protein has a half-life of ≈36 hr (data not shown). This long half-life suggests that regulation of NF1 occurs in ways other than changes in NF1 protein level.

NF1 Protein Is Present in a Large Molecular Mass Complex. To examine the possible association of NF1 with other cellular protein(s), a glycerol gradient/immunoprecipitation analysis was carried out. Cells were lysed under mild conditions in the presence of glycerol, and lysates were centrifuged through a glycerol gradient, which was then fractionated. Aliquots of each fraction were then subjected to immunoprecipitation with preimmune serum and anti-NF1 antiserum (Fig. 4). Although some NF1 was found in fractions expected for the 280-kDa monomers, the majority of NF1 was recovered in fractions that correspond to molecular masses that are much larger, on the order of 600–800 kDa (Fig. 4*A*, lanes 1–4). In addition, we noted the presence of a 400- to 500-kDa protein in the same fractions as NF1 and only in the lanes immunoprecipitated with immune antiserum (Fig. 4*A*, arrowhead). The same 400- to 500-kDa band was also detected in immunoprecipitates when cells were lysed under the mild conditions described above but the extracts were

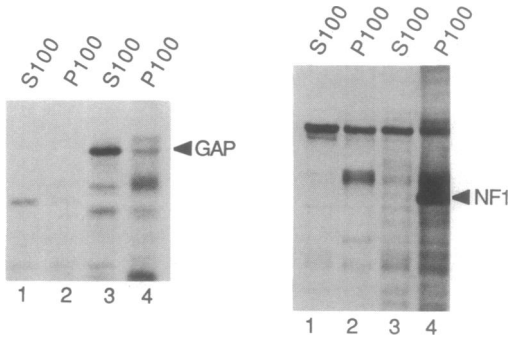

FIG. 3. Intracellular localization of 280-kDa NF1 protein and GAP in NIH 3T3 cells as determined by biochemical subcellular fractionation. Cells were labeled and lysed by Dounce homogenization as described, and the postnuclear supernatant was centrifuged at 100,000 × *g* to yield the supernatant (S100) and pellet (P100) fractions. Equal portions of these fractions were subjected to immunoprecipitation with normal rabbit serum (lanes 1 and 2), anti-GAP (*Left*, lanes 3 and 4), or anti-NF1 (*Right*, lanes 3 and 4) antiserum and analyzed on 6% gels.

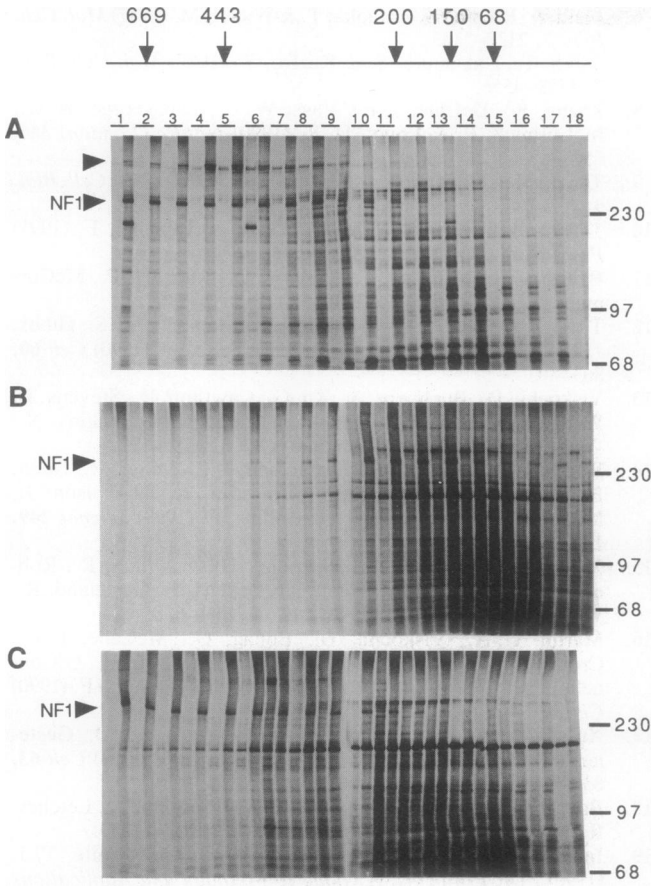

FIG. 4. NF1 protein is present in a large molecular mass complex in NIH 3T3 cells, as demonstrated by glycerol gradient centrifugation. (*A*) Native complex. (*B*) Cell lysate boiled before gradient centrifugation. (*C*) Gradient fractions boiled after centrifugation and before immunoprecipitation. Cells were labeled and lysed, and the lysates were centrifuged as described. Fractions (numbered 1–18, with the most dense fraction on the left) were removed, split into two, and immunoprecipitated with either preimmune (first lane of each fraction) or immune serum 31 (second lane of each fraction). Arrowheads on the left denote location of 400- to 500-kDa protein (top arrowhead) and NF1 (arrowheads labeled NF1). Arrows below numbers at the top denote the peak migration of calibration standards through the gradient; numbers on the right denote migration of molecular mass markers (kDa) through the 6% gels.

immunoprecipitated directly without prior gradient centrifugation (data not shown).

To rule out the possibility that the coprecipitating 400- to 500-kDa band might be recognized directly by the antiserum, the gradient fractions were boiled with SDS and reducing agent prior to immunoprecipitation. Under these conditions, NF1 was recovered in the dense fractions, as expected, but the 400- to 500-kDa protein was absent from the immunoprecipitates (Fig. 4*C*). This result makes it unlikely that the 400- to 500-kDa protein is recognized by the anti-NF1 antiserum or represents an NF1 dimer, which therefore strengthens the possibility that the protein appears in the immunoprecipitates via specific association with NF1.

It was also possible that the NF1 protein in the large molecular mass fractions might simply be migrating aberrantly. To examine this possibility, a cell lysate was boiled in the presence of SDS and reducing agent prior to glycerol gradient sedimentation. Following this protocol, all of the NF1 was recovered in fractions corresponding to the subunit molecular mass of NF1 (Fig. 4*B*, lanes 9–12), and the 400- to 500-kDa protein was absent. This observation verifies that the presence of NF1 in large molecular mass fractions as shown in Fig. 4*A* is due to the formation of a complex with itself and/or other proteins.

When a similar analysis was carried out by using lysates from v-*src* transformed cells or HeLa cells, the same pattern of NF1 sedimentation and association with the 400- to 500-kDa protein was observed (data not shown). The pattern of NF1 sedimentation in a rat Schwannoma line, RN-22 [Fig. 5*B*, provided by Nancy Ratner (25)] was compared to that found in NIH 3T3 cells (Fig. 5*A*). In the RN-22 line, NF1 was also present in large molecular mass fractions; however, much less of the 400- to 500-kDa protein was recovered (Fig. 5*B*, arrowhead). We conclude that either the nature of the NF1 complex(es) in the Schwannoma cells is different, or the association of the 400- to 500-kDa protein with NF1 is more labile and does not survive the washes and processing of the samples prior to gel electrophoresis.

DISCUSSION

In this study, we have identified the NF1 protein in mammalian cells and have characterized some of its properties. The difference in the subcellular locations of NF1 and GAP implies that both newly formed cytosolic $p21^{ras}$ protein as well as membrane-associated $p21^{ras}$ can be negatively regulated (26–28). When $p21^{ras}$ is synthesized in the cytosol, it is probably placed initially in the active form because of the high intracellular concentration of GTP (5). Even when $p21^{ras}$ is membrane bound, its catalytic region, which interacts with GAP and also, presumably, with NF1, must remain accessible to the cytosol so it can efficiently exchange bound GDP with GTP in response to an upstream signal (29–32).

The two immune antisera generated against the NF1-GRD recognize partially nonoverlapping epitopes, since one efficiently neutralizes the enzymatic activity of NF1-GRD, while the other does not. Although both sera detected the same 280-kDa protein in mammalian cell lysates, the serum with neutralizing activity was less efficient. This latter result may

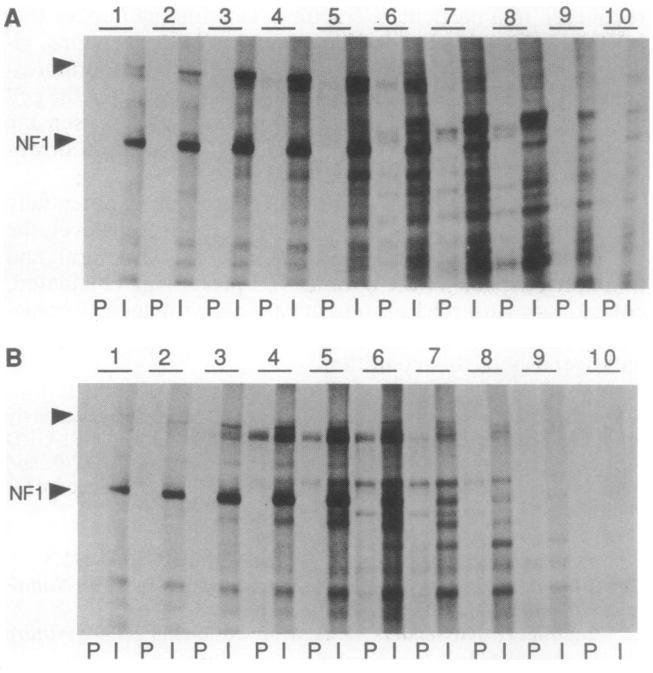

FIG. 5. NF1 large molecular mass complex is found in NIH 3T3 cells (*A*) and a rat Schwannoma cell line (RN-22) (*B*). Experiment was performed as described in Fig. 4, except that each gradient was divided into only 10 fractions (numbered 1–10 at the top). Each fraction was subjected to immunoprecipitation with preimmune (P) or immune (I) serum. Arrowheads on the left denote location of 400- to 500-kDa protein (top arrowhead) and NF1 (labeled NF1).

suggest that the NF1 protein is complexed *in vivo* with another molecule such that formation of the complex inhibits NF1 binding by the serum with neutralizing activity.

In support of this possibility, NF1 protein in rodent and human cells was apparently associated with a 400- to 500-kDa protein in a large molecular mass complex, which could be disrupted by boiling. This finding may suggest that NF1 is regulated in part through these associations, for a significant proportion of the cellular NF1 does appear to be a monomer. The observation that much less of the 400- to 500-kDa protein was found associated with the NF1 protein in the rat Schwannoma cell line may indicate that the nature of the complex is different in this cell type. This possibility is provocative, since Schwann cells appear to be the major target for phenotypic abnormalities in patients with NF1.

In yeast, *IRA1* is reported to be required for adenylate cyclase, which is a major downstream target of yeast *RAS*, to properly localize to the membrane, perhaps via a direct interaction between IRA1 protein and cyclase (33). It is therefore tempting to speculate that the large molecular mass protein associated with NF1 might represent a downstream target of mammalian *ras*, in view of the extensive sequence homology between the IRA1 and NF1 protein products.

Several reports using NF1-GRD have emphasized its similarities with GAP, although differential sensitivity to several compounds has been shown for their GTPase-accelerating activities (34, 35). Here we have described other potentially important ways in which NF1 and GAP differ. GAP is found mainly in the cytoplasm under steady-state conditions (5). However, phosphorylation of GAP on tyrosine residues after activation of receptor tyrosine kinases or transformation by nonreceptor tyrosine kinases results in a portion of GAP shifting to the cell plasma membrane and complexing with a 62-kDa protein (21, 36). By contrast, NF1 is not phosphorylated at tyrosine in v-*src*-transformed cells, nor was there a difference in the NF1 large molecular mass complex in these cells. Furthermore, the NF1 protein, unlike GAP, was recovered in a particulate fraction. This finding implies that NF1 may be associated with an intracellular structure, although no clear association was detected by immunofluorescence. These differences in GAP and NF1 may allow the cell to regulate $p21^{ras}$ in a more elaborate way, by sensing incoming signals as well as providing constitutive downmodulation of $p21^{ras}$ function.

Identification of the NF1 protein represents a potentially important step toward understanding, at the protein level, the molecular basis of neurofibromatosis. As structural and functional aspects of the normal NF1 protein are elucidated, comparison with NF1 protein in patients with neurofibromatosis may provide insights into the molecular pathology and pathogenesis of this condition.

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