COOH-terminal SH2 domain were used for the excision. Src and Crk fusion proteins utilized natural restriction sites. The resulting fusion proteins contained the NH_2-terminal 323 amino acids of TrpE and retained the desired reading frame for $PLC_\gamma 1$ or GAP.

20. Cultures of *E. coli* RR1 with pATH expression plasmids were grown, induced, and lysed as described (*29*). The TrpE fusion proteins were recovered from the supernatants by immunoprecipitation with polyclonal anti-TrpE antiserum immobilized on protein A-Sepharose beads. Immune complexes were washed (*29*), aliquoted, flash-frozen, and stored at −70°C until mixed with mammalian cell lysates. Starved or growth factor-stimulated rat fibroblasts (~5×10^6) were lysed in 2 ml of lysis buffer (50 mM Hepes, pH 7.0, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 100 mM NaF, 10 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Clarified mammalian cell lysate (1 ml) was mixed with immobilized bacterial fusion protein by gentle inversion for 90 min at 4°C. Complexes were recovered by centrifugation, washed three times with HNTG buffer (20 mM Hepes pH 7.0, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, 1 mM Na_3VO_4), and analyzed by immunoblotting with anti-P.Tyr or anti-receptor as described (*6, 15, 18, 29*). To ensure that the different TrpE fusion proteins were present in similar amounts in the immune complexes incubated with the mammalian cell lysates, duplicate samples for anti-P.Tyr and anti-EGF-R immunoblotting were probed with an anti-TrpE monoclonal antibody. Equivalent amounts of the various TrpE fusion proteins were detected.
21. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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32. Bacterial fusion proteins contained the following amino acid residues: Src-SH3-SH2, $p60^{v\text{-}src}$ 87 to 291; PLC-SH2[N], bovine $PLC_\gamma 1$ 547 to 659; PLC-SH2[C], $PLC_\gamma 1$ 663 to 752; PLC-SH2[N + C], $PLC_\gamma 1$ 547 to 752; PLC ΔSH2-SH2-3, $PLC_\gamma 1$, 555 to 951; PLC-SH3, $PLC_\gamma 1$ 781 to 855; GAP-SH2[N], human GAP 178 to 277; GAP-SH2[C], GAP 348 to 444; GAP-SH2[N + 3 + C], GAP 178 to 444; Crk-SH2, $p47^{gag\text{-}crk}$ 205 to 386; Crk-SH2-SH3, $p47^{gag\text{-}crk}$ 220 to 427.
33. Equivalent contributions were made by the first two authors. We thank M. Hoffmann, J. Knopf, and G. S. Martin for critical review of the manuscript; B. Margolis and J. Schlessinger for anti-EGF-R antibodies; S. A. Courtneidge for anti-PDGF-R antibodies; K. Letwin for anti-P.Tyr antibodies; J. Knopf for bovine $PLC_\gamma 1$ cDNA; F. McCormick for human GAP cDNA; H. Hanafusa for v-*crk* DNA; J. M. Bishop for SR-A RSV v-*src* DNA; and M. Weber for R1hER cells. Supported by grants from the National Cancer Institute of Canada (NCIC) and the Medical Research Council of Canada (MRC). D.A. and M.F.M. are postdoctoral fellows of the NCIC. T.P. is a Terry Fox Cancer Research Scientist of the NCIC. C.A.K. is a graduate fellow of the MRC.

17 July 1990; accepted 22 August 1990

A Cytoplasmic Protein Inhibits the GTPase Activity of H-Ras in a Phospholipid-Dependent Manner

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A cytoplasmic protein has been identified that inhibits the guanosine triphosphatase (GTPase) activity of bacterially synthesized, cellular H-Ras protein. This GTPase inhibiting protein is able to counteract the activity of GTPase activating protein (GAP), which has been postulated to function as a negative regulator of Ras activity. The potential biological importance of the GTPase inhibiting protein is further supported by its interaction with lipids. Phospholipids produced in cells as a consequence of mitogenic stimulation increase the activity of the GTPase inhibiting protein, as well as inhibit the activity of GAP. The interaction of such lipids with each of these two regulatory proteins would, therefore, tend to increase the biological activity of Ras and stimulate cell proliferation.

ALTHOUGH IT APPEARS THAT CELLULAR Ras proteins are important in proliferative signal transduction (*1, 2*), the mechanism by which Ras is regulated remains unclear. As with other guanine nucleotide–binding proteins, Ras is thought to be biologically active when bound to guanosine triphosphate (GTP); its inherent GTPase activity, however, slowly converts Ras-GTP to Ras-GDP (guanosine diphosphate), which is thought to be biologically inactive. A change in either the nucleotide exchange rate of Ras or the inherent GTP hydrolysis rate could therefore be responsible for controlling the biological activity of the protein. A GTPase activating protein (GAP) has been identified (*3–5*) that stimulates the GTPase activity of normal Ras more than 100-fold without affecting oncogenic mutant Ras proteins. Because GAP can stimulate the conversion of Ras-GTP into Ras-GDP, it could function in suppressing the biological activity of Ras (*6*). On the other hand, on the basis of mutation analysis indicating that GAP interacts with a sequence that is essential for Ras biological activity (*3, 4*), it has been suggested that GAP might act as the Ras effector protein.

On the basis of results of microinjection experiments, we previously suggested that the biological activity of Ras could be controlled by phospholipids (*7, 8*). This was supported by our observation that GAP is inhibited by certain phospholipids in vitro (*8*), and we further demonstrated that GAP physically associates with lipids including phosphatidic acid (PA), phosphatidylinositol monophosphate, and arachidonic acid; other lipids were ineffective (*9*). The physical association between GAP and phospholipids resulted in the retention of GAP on a lipid affinity column. GAP bound to such a PA column was released after treatment with EDTA, suggesting that its association with lipid requires divalent cations. When a preparation of soluble, cytoplasmic proteins was passed over a PA-containing affinity column and then eluted with EDTA, two protein fractions with opposite effect on Ras GTPase activity were obtained: one of these fractions contained GAP and stimulated GTPase, whereas the second fraction inhibited Ras GTPase activity. We have now characterized this GTPase inhibitor.

A mouse brain extract that had been eluted from DEAE-Sephacel was added to a PA affinity column. After extensive washing (Fig. 1), the column was then eluted with 10 mM EDTA. The GTPase activity of H-Ras was inhibited by eluted fractions 1 to 3 (*10*) (Fig. 1A). Later fractions eluted from the same column contained GAP (*9*). Thus, there were two distinct enzymatic activities in mouse brain extract that bind to PA in a cation-dependent manner. One is GAP, whereas the other is a GTPase inhibiting protein. We confirmed these results, which were observed with a filter binding assay (*11*) (Fig. 1A), by immunoprecipitation analysis (Fig. 1B) (*12*).

In the immunoprecipitation assay, an in-

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creased rate of nucleotide exchange would result in the increased binding of Ras to [α-^{32}P]GTP, giving the appearance of a decrease in GTPase activity. To rule out the possibility that increased nucleotide exchange was responsible for the observed decrease in GTPase activity in the presence of the inhibitory protein fractions, a 600-fold excess of unlabeled GTP was added to the reaction mixture at the time of addition of Mg^{2+}. Nucleotide exchange would thus result in the replacement of ^{32}P-labeled GTP by unlabeled GTP and a decrease in the overall labeling of Ras. Under these conditions, the total labeled nucleotide bound to immunoprecipitated Ras was not altered by the inhibitory protein fractions from the affinity column. This indicates that GTPase activity rather than exchange rate is altered. This conclusion was confirmed by results with the filter binding assay, in which an excess of unlabeled GTP is routinely included. Increased nucleotide exchange would therefore result in decreased protein-associated ^{32}P, whereas inhibition of GTPase activity would have the opposite effect. Results from both assays show that the activity identified inhibits the basal level of Ras GTPase activity. No alteration in the mobility of Ras on an SDS-polyacrylamide gel or evidence of phosphorylation of Ras has been observed as a consequence ofGTPase inhibiting protein activity.

The GTPase inhibitor isolated from the affinity column has an apparent molecule mass of 60,000 daltons on a Sephadex G-75 molecular exclusion column. We do not know if this protein is similar to any of the proteins of similar size that associate with GAP (*13*). The inhibitory activity was completely abolished by treatment with chymotrypsin, indicating that the inhibitor is a protein. It is a relatively heat-stable protein because boiling for 10 and 15 min causes only 30% and 60% loss of activity, respectively. To more thoroughly characterize the GTPase inhibiting protein, we partially purified it from bovine brain by a procedure including DEAE-Sephacel, a PA affinity column, and a Sephadex G-75 size exclusion column, resulting in a 500-fold purification. This material was used for all analyses presented below, but the preparation was concurrently further purified by heat treatment and high-performance liquid chromatography on a DEAE resin, resulting in an overall 10,000-fold purification of the GTPase inhibiting protein (*14*). Results presented with partially purified material were repeated with the highly purified protein. There was no apparent biochemical difference between the properties of the partially and highly purified GTPase inhibiting protein.

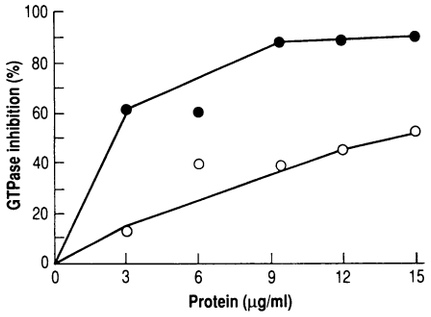

Fig. 2. Dose response and lipid enhancement of GTPase inhibition. Ras was allowed to associate with [γ-^{32}P]GTP and then incubated with increasing amounts of GTPase inhibiting protein in the presence (●) or absence (○) of 1-stearoyl, 2-arachidonoyl PA (80 μg/ml). In the presence of lipid, the GTPase inhibiting protein caused a maximal inhibition of ~90%. Inhibition of GTPase activity was calculated from the filter binding assay as follows: Z = the binding of labeled GTP at zero time; Y = the binding after 60 min at 30°C in the absence of added material; X = the binding after incubation together with added GTPase inhibiting protein with or without added lipid; percentage inhibition = $[(X - Y)/(Z - Y)] \times 100\%$. Similar results were obtained with the immunoprecipitation assay. Results are from a single experiment, but are representative of two other experiments.

In the absence of phospholipid, GTPase inhibiting protein inhibited Ras GTPase in a dose-dependent manner (Fig. 2). Furthermore, the activity of the GTPase inhibiting protein was stimulated twofold by 0.15 M NaCl. This concentration of NaCl inhibits the ability of GAP to stimulate the GTPase activity of Ras. In time course studies, the inhibitory factor reduced the rate of GTPase at all times tested between 5 and 50 min. Finally, no GTP binding activity could be detected in the partially or highly purified preparations of the inhibitory factor.

The GTPase inhibiting protein was first identified by virtue of its binding to a PA affinity column, indicating that this protein, like GAP, binds phospholipids. Those lipids able to bind GAP block its ability to stimulate Ras GTPase activity (*8*). Therefore, we tested whether phospholipids were also able to alter the activity of GTPase inhibiting protein. H-Ras protein bound to [γ-^{32}P]GTP was incubated with GTPase inhibiting protein along with 1-stearoyl, 2-arachidonoyl PA (80 μg/ml) in the presence of 0.15 M NaCl and 3 mM $MgCl_2$. In this reaction, the ability of GTPase inhibiting protein to inhibit GTPase activity was enhanced up to threefold compared to reactions without added lipid (Fig. 2). Thus, PA affects GAP and the GTPase inhibiting protein in opposite directions; GAP activity is inhibited, whereas GTPase inhibiting protein is stimulated. In both cases, the result of these lipid interactions is to increase the proportion of Ras associated with GTP and thus to increase its biological activity. PA itself had no direct effect on the GTPase activity of Ras.

A series of phospholipids was tested for their ability to stimulate the GTPase inhibiting protein. Among the phospholipids with the highest activity were those affected metabolically by mitogenic stimulation, in-

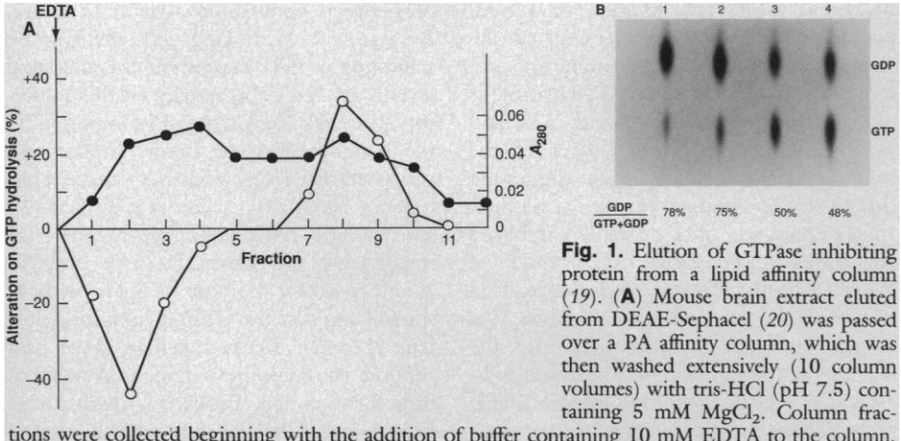

Fig. 1. Elution of GTPase inhibiting protein from a lipid affinity column (*19*). (**A**) Mouse brain extract eluted from DEAE-Sephacel (*20*) was passed over a PA affinity column, which was then washed extensively (10 column volumes) with tris-HCl (pH 7.5) containing 5 mM $MgCl_2$. Column fractions were collected beginning with the addition of buffer containing 10 mM EDTA to the column. Each fraction was then assayed by the filter binding method (*11*) for its effect on Ras GTPase activity. Alteration in GTP hydrolysis (○) is calculated by comparing the amount of ^{32}P remaining associated with Ras in the presence (P) or absence (A) of added factors. Percent alteration equals $[(A - P)/A] \times 100\%$. The basal GTP hydrolysis rate in the assay is 1.2 pmol/liter per hour at 30°C with 128 ng of Ras. The absorbance at 280 nm (A_{280}) (●) of each fraction is also shown. (**B**) Inhibition of Ras GTPase activity by the factor eluted from the lipid affinity column. Fractions 1 to 3 were further tested for their ability to inhibit Ras GTPase activity with the immunoprecipitation GTPase assay (*12*). The ratio of the amount of ^{32}P present on GDP to that present in GDP plus GTP indicates the extent of GTP hydrolysis. Lanes 1 and 2, Ras protein without added column fractions. Lanes 3 and 4, Ras plus protein (20 μg/ml) from the combined fractions 1 to 3. In both (A) and (B), EDTA present in the column fractions may inhibit the basal rate of Ras GTPase. Data are from a single analysis but are representative of results obtained in repeated experiments.

cluding PA, phosphatidylinositol monophosphate, arachidonic acid, and diacylglycerol. Phospholipids that are major structural components of the plasma membrane—such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine—had only a slight effect, which was independent of lipid concentration. Diacylglycerol (dilinolein), which induced a fourfold stimulation of GTPase inhibiting protein activity, was the most potent, both in the amount required for stimulation and in the overall degree of stimulation. Dilinolein was followed by PA, arachidonic acid, and phosphatidylinositol monophosphate, which stimulated activity three-, two-, and 1.5-fold, respectively (Fig. 3). Among phosphatidic acids, only those containing unsaturated fatty acids [such as arachidonic acid (20:4)] had potent stimulating effects. Except for diacylglycerol, the lipids that strongly enhanced GTPase inhibiting protein activity were those with the strongest ability to inhibit GAP (*8*). Highly purified GTPase inhibiting protein was stimulated by phospholipids to the same extent as the partially purified material.

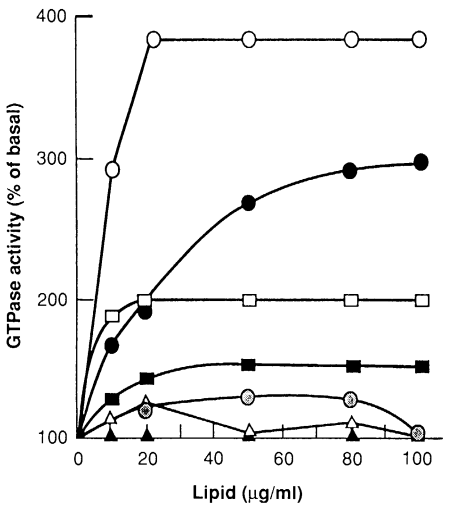

Fig. 3. Enhancement of GTPase inhibiting activity by various lipids. Lipids were prepared as liposomes, as described for PA (*19*). GTPase inhibiting protein (9 μg/ml) was added to each reaction along with various concentrations of each type of lipid. After incubation for 60 min at 30°C, the extent of GTP hydrolysis was determined by filter binding. Dilinolein (○), 1-stearoyl, 2-arachidonoyl PA (●), arachidonic acid (□), phosphatidylinositol monophosphate (■). Although some inhibition by more common structural phospholipids was observed, this limited inhibition was not observed to be dose-dependent and its relevance is therefore unclear. These lipids included phosphatidylethanolamine (dotted circles), phosphatidylcholine (△), and phosphatidylserine (▲). Percentage of basal GTPase activity was calculated as the inhibition (calculated as in Fig. 2) observed in the presence of added lipid as a percentage of the inhibition obtained without added lipid. Results are from a single experiment, but are representative of two other experiments.

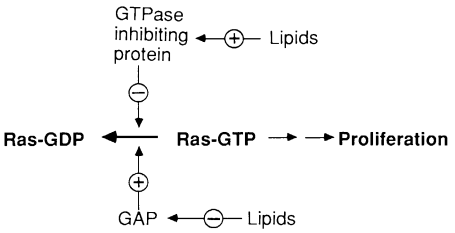

Fig. 4. Model for the role of lipids in the control of Ras activity. Evidence indicates that Ras proteins are able to function biologically to transmit a proliferative signal only when bound to GTP. GAP would therefore tend to reduce the biological activity of Ras by increasing the rate of GTPase activity. In the presence of lipids produced as a result of mitogen stimulation this activity would be blocked. GTPase inhibiting protein, on the other hand, inhibits the GTPase activity of Ras, resulting in an increase in the proportion of Ras bound to GTP and therefore an increase on biological activity. This effect is enhanced in the presence of lipids. Lipids, therefore, are postulated to affect both of these regulatory proteins so as to increase the biological activity of Ras.

When crude cytoplasmic extracts are incubated with Ras, GAP activity predominates over the GTPase inhibitory activity described here, resulting in a marked stimulation in the rate of Ras GTPase. When enough partially purified GTPase inhibiting protein is added, however, GAP activity can be counteracted, resulting in a net decrease in GTP hydrolysis. We tested the ability of partially purified GTPase inhibiting protein (8 μg/ml) to inhibit GAP activity in a crude cytoplasmic extract. The basal rate of GTPase activity resulted in a 33% hydrolysis of bound GTP. The addition of a GAP preparation increased this to 64% hydrolysis, whereas added GTPase inhibitory protein (without GAP) reduced GTP hydrolysis to 20%. In the presence of both GAP and GTPase inhibiting protein, 46% of the bound GTP was hydrolyzed, suggesting that the two proteins counteract each other. In the presence of a partially inhibitory concentration of 1-stearoyl, 2-arachidonyl PA (30 μg/ml), GTP hydrolysis was reduced to 50% in the presence of GAP but to 28% in the presence of both GAP and the GTPase inhibiting protein. This result indicates that GTPase inhibiting protein is able to overcome the effect of GAP in the presence of a lipid concentration too low to efficiently inhibit GAP activity in the absence of the inhibitory protein. The effect of GTPase inhibiting protein, therefore, is more pronounced in the presence of stimulatory lipids.

To characterize the interaction between Ras and the GTPase inhibitory protein, we carried out a competition assay. Standard assays with Ras bound to labeled nucleotide were performed in the presence or absence of a fourfold excess of Ras bound to unlabeled nucleotide [0.5 μM; along with bovine serum albumin (300 μg/ml) as a carrier]. In the presence of excess Ras bound to unlabeled GTP or GDP, the activity of GTPase inhibiting protein was reduced by 75 and 74%, respectively, compared to those reactions without added, competing Ras protein. This type of substrate competition shows that there is a direct interaction between Ras and GTPase inhibiting protein, and that GTPase inhibiting protein is able to associate with Ras bound either to GDP or GTP. For comparison, GAP associates only with Ras bound to GTP (*15*). The mechanism by which the GTPase activity of Ras is inhibited by the GTPase inhibitory protein is unknown. Stoichiometric analysis indicated that the quantity of Ras in the assay exceeds that of GTPase inhibiting protein by at least 50-fold. It seems possible, therefore, that the inhibitory protein induces an alteration in the conformation of Ras that inhibits its GTPase activity and which persists for some time after the two proteins separate.

The phospholipids that inhibit GAP and activate GTPase inhibiting protein are the phospholipids actively produced in many cells by serum or growth factor stimulation (*16*). The inhibition of GAP activity by these lipids would tend to increase the proportion of Ras bound to GTP and thus its biological activity. The stimulation by these lipids of the GTPase inhibiting protein would have the same effect on the biological activity of Ras (Fig. 4). The lipids produced as a result of mitogen stimulation would, therefore, interact with both GAP and the GTPase inhibiting protein to promote the biological activity of Ras. The activity of this inhibitory protein is likely to be most important in the presence of these lipids. It has been proposed that tyrosine kinases initiate a mitogenic signal that is received by Ras (*1*). Interestingly, tyrosine kinases interact both functionally and physically with enzymes able to produce the type of lipids we have shown stimulate the GTPase inhibiting protein (*17, 18*). Taken together, these data support the hypothesis that lipid metabolism forms a link between growth factor receptors and cellular Ras proteins that is critical in the control of proliferation.

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10. The elution of the two protein fractions with the ability to alter Ras GTPase activity from a PA affinity column seems to depend on the size of the column. In our previous experiments, when a small PA affinity column (2 ml) was used, only GAP activity was detected. However, in the present study, a large PA affinity column (5 ml) allows the separation of both GAP and GTPase inhibiting protein activities. The two proteins are thus apparently not separable by the smaller PA affinity column.
11. For filter binding assays, purified bacterially synthesized Ras was first loaded with [γ-^{32}P]GTP by incubation with 20 μl of incubation buffer containing 20 mM tris-HCl (pH 7.5), 1 mM dithiothreitol, and 2 μM [γ-^{32}P]GTP (10 Ci/mmol; Amersham) at 30°C for 10 min. GTPase activity was then assayed by incubating the [γ-^{32}P]GTP-bound Ras (0.1 μM) at 30°C for 60 min in 100 μl of reaction buffer [20 mM tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM $MgCl_2$, 1 mM GTP]. The reaction mixture was then applied to nitrocellulose filters (0.45 μm, 25 mm; BA 85, Schleicher & Schuell), which were immediately washed with 10 ml of 20 mM tris-HCl (pH 7.5) containing 5 mM $MgCl_2$ and 50 mM NaCl. The amount of [γ-^{32}P]GTP remaining on the filters, which reflects the GTPase activity of Ras, was determined. The data described here obtained with renatured Ras protein, but similar results have been obtained with Ras prepared in soluble form, 70 to 80% of which binds nucleotide.
12. Nucleotide-free Ras (0.2 μM) was incubated for 20 min at 30°C with 1 μM [α-^{32}P]GTP (3000 Ci/mmol; Amersham) in 50 μl of tris-HCl (pH 7.5) containing 2 mM dithiothreitol. The GTPase reaction was initiated by addition of $MgCl_2$ with or without test samples in 100 μl of reaction buffer [final concentrations: 20 mM tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.15 M NaCl, 1 mM dithiothreitol]. After incubation at 30°C for 1 hour, Ras was immunoprecipitated by monoclonal antibody Y13-259 and protein A–Sepharose beads coated with rabbit antibody to rat immunoglobulin G. Bound nucleotides were released from the immunoprecipitate by boiling for 3 min, resolved on polyethyleneimine cellulose thin-layer chromatography plates (EM Science) in 1 mM potassium phosphate (pH 3.4), and visualized by autoradiography. Ras protein was prepared in a bacterial expression system.
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19. An affinity column (5 ml) containing immobilized 1-stearoyl, 2-arachonoyl PA was prepared by first chemically linking arachidic acid (20:0) (prepared as liposomes in acetate buffer) to agarose gel containing a six-atom hydrophilic spacer arm (AFF-gel 102; Bio-Rad). The covalently attached fatty acid is unavailable for association with GAP (*9*), but it was able to associate with and retain subsequently added liposomes, presumably by hydrophobic interactions. Liposomes of PA were passed over the column to generate the affinity column used. PA liposomes were prepared by dissolving PA (0.5 to 1.0 mg; Sigma) in chloroform, followed by drying under vacuum, in a glass tube (12 by 75 cm). The thin layer of lipid coating the glass was suspended in 1 ml of 0.1 M tris-HCl (pH 7.5) and sonicated in an ice bath for 30 s by inserting a titanium microtip (Fisher, Model 300 Sonic Dismembrator) into the bottom of the glass tube. Half-maximum power output was used for liposome-micelle preparation.
20. The cerebra from ten mice were homogenized in ice-cold hypotonic buffer [10 mM tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride]. The homogenate was centrifuged at 3000*g* for 10 min to remove unbroken cells, and resulting supernatant was then centrifuged at 100,000*g* for 30 min. The supernatant (5 mg of protein per milliliter), which contained GTPase inhibiting protein activity, was stored at −80°C. Before application to the PA affinity column, the preparation (30 ml) was incubated with 10 ml of DEAE-Sephacel (Pharmacia LKB) for at least 2 hours in tris-HCl (pH 7.5) containing 5 mM $MgCl_2$ and 1 mM EGTA. After washing the resin with 1 liter of the same buffer five times, proteins were eluted from the resin by the same buffer containing 0.3M NaCl.
21. We thank M. Roudebush, M. Golubic, and S. Dobrowolski for advice and technical assistance, and J. Leis and D. R. Lowy for helpful reviews of the work and manuscript. Supported in part by a grant (CA 48662) from the NIH and by the DeBartolo Fund.

8 June 1990; accepted 7 August 1990

Mesodermal Control of Neural Cell Identity: Floor Plate Induction by the Notochord

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The floor plate is a specialized group of midline neuroepithelial cells that appears to regulate cell differentiation and axonal growth in the developing vertebrate nervous system. A floor plate–specific chemoattractant was used as a marker to examine the role of the notochord in avian floor plate development. Expression of this chemoattractant in lateral cells of the neural plate and neural tube was induced by an ectopic notochord, and midline neural tube cells did not express the chemoattractant after removal of the notochord early in development. These results provide evidence that a local signal from the notochord induces the functional properties of the floor plate.

THE DEVELOPMENT OF THE VERTEbrate nervous system begins with the induction of the neural plate from undifferentiated ectoderm in response to signals that derive from adjacent mesoderm (*1, 2*). Within the neural plate, the first cells to differentiate are located at its midline (*3*) and give rise to the floor plate, a distinct structure at the ventral midline of the neural tube (*4, 5*). The floor plate appears to be the source of a signal that regulates the pattern of cell differentiation along the dorsoventral axis of the neural tube (*6, 7*). The floor plate also contributes to axonal patterning by releasing a diffusible factor that may attract commissural axons to the ventral midline of the spinal cord (*8*) and by guiding these axons after they cross the midline (*9*). The specialized functions of the floor plate have led us to examine the interactions that control its differentiation.

Prospective floor plate cells are located immediately above the notochord, and notochord grafts placed next to the neural tube cause wedging of the adjacent neural epithelium similar to that observed during the early development of the floor plate (*10*). These results have led to the suggestion that floor plate differentiation is induced by the notochord (*10, 11*). However, comparable changes in neuroepithelial cell shape occur in many regions of the developing nervous system (*12*); thus it is not possible unambiguously to identify the floor plate by its morphology. The floor plate–specific chemoattractant (*8*) provides a marker with which to detect the floor plate and its development in response to the notochord. Here, we report that this chemoattractant is induced in lateral cells of the chick neural plate and neural tube in vivo and in vitro and that the chemoattractant does not appear in cells at the ventral midline of the neural tube in the absence of the notochord. A local signal from the notochord therefore appears to act on overlying neural plate cells to induce floor plate differentiation.

To study the induction of the floor plate in the chick embryo, we initially examined whether chick floor plate causes outgrowth and orientation of commissural axons from explants of E11 rat dorsal spinal cord (*13, 14*). Production of the chemoattractant was quantified by counting the number of axon bundles that emerged from the rat dorsal explants. Floor plate explants from stage 6

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