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22. After incubation, micelles were run on a 0.7 cm by 50 cm column of Sephacryl S-300 at 4°C. SUVs (PI) and LUVETs were run on a 0.7 cm by 10 cm or 0.7 cm by 26 cm column of Sephadex G-100 at room temperature. Both columns were equilibrated with sample buffer, and the flow rate was 20 ml/hour. Fractions of 0.4 to 0.6 ml were assayed for protein [M. Bradford, *Anal. Biochem.* **72**, 248 (1976)]. Because lipids quench the Bradford dye-binding assay, the data in Fig. 1, A to D, are given in arbitrary units. The fraction of bound profilin was calculated as the difference between the total amount applied to the column and the amount in the entire included peak of free protein (centered on fraction 45 in Fig. 1, A to D).
23. Profilin was purified from outdated human platelets by affinity chromatography on poly-L-proline–Sepharose (*16*). Actin was purified from rabbit skeletal muscle [J. A. Spudich and S. Watt, *J. Biol. Chem.* **246**, 4866 (1971); T. D. Pollard, *J. Cell Biol.* **103**, 2747 (1986)] and dialyzed in buffer B [5 m*M* tris (*p*H 7.5), 75 m*M* KCl, 0.5 m*M* dithiothreitol, and 0.1 m*M* NaN_3] before the PLC assay. Ovalbumin (Sigma) was dissolved in buffer B.
24. The purity of the phosphoinositides PI, PIP, and PIP_2 was confirmed by thin-layer chromatography with 10 μg of each lipid loaded onto silica gel 60 plates in a 90:90:7:22 chloroform, methanol, ammonium hydroxide, and water solvent system. Homogeneous PIP_2 micelles were prepared by suspending 1 mg of PIP_2 (Calbiochem) in 1 ml of deionized water and sonicating in a Bransonic 32 (bath-type) sonicator for 5 min at room temperature. PI (Sigma) SUVs were prepared in the same manner as PIP_2 micelles. Large unilamellar vesicles of various composition were obtained by the extrusion technique (*7*). Mixtures of lipids were dried in a glass tube under a stream of nitrogen and were then resuspended in deionized water (0.5 to 1.2 ml) by vortexing. The PC, PS, and PE were obtained in chloroform (Avanti Polar Lipids, Pelham, AL). [2-^{3}H]Phosphatidyl inositol 4,5-bisphosphate ([^{3}H]PIP_2) was obtained in dichloromethane, ethanol, and water (20:10:1) (Amersham). After five cycles of freezing in liquid nitrogen and thawing in a water bath (at 36°C), samples were passed ten times through a filter (polycarbonate, 0.1 μm pore size, Nuclepore) in the extruder under a pressure of 400 psi. The concentration of lipid in each mixture was measured by liquid scintillation counting of a portion of the sample after extrusion.
25. For the PLC assay, [^{3}H]PIP_2 (final specific activity of 0.05 to 0.10 Ci/mol) was mixed with unlabeled PIP_2 to form micelles or with unlabeled PIP_2 and other lipids to form LUVETs of known composition (*24*). Hydrolysis was stopped by addition to the samples (100 μl) of ice-cold methanol, chloroform, and HCl [(3:1:1), 625 μl], which results in the separation of IP_3 from lipids. The [^{3}H]IP_3 in the aqueous phase was measured by liquid scintillation counting [M. G. Low and W. B. Weglicki, *Biochem. J.* **215**, 325 (1983); J. J. Baldassare and G. J. Fisher, *J. Biol. Chem.* **261**, 11942 (1986); D. M. Raben, K. Y. Yasuda, D. D. Cunningham, *Biochemistry* **26**, 2759 (1987)]. Because the PIP_2 concentration (S) was much smaller than the K_m of the enzyme (*9*), the rate of hydrolysis (v) was directly proportional to the PIP_2 concentration according to the Michaelis-Menten equation $v = V_{max} S/K_m + S$, where V_{max} is the maximal rate of hydrolysis. The effect of PIP_2 sequestration by profilin on the hydrolysis rate can be directly calculated from the Michaelis-Menten equation where S is replaced by: $1/2\{[(K_d + P_T - S_T)^2 + 4K_d S_T]^{1/2} - (K_d + P_T - S_T)\}$, where P_T and S_T are the total profilin and total PIP_2 concentrations, respectively, and K_d is the dissociation constant for the profilin-PIP_2 complex.
26. To add PIP_2 to the outer leaflet of LUVETs [P. A. Janmey and T. P. Stossel, *J. Biol. Chem.* **264**, 4825 (1989)], we incubated LUVETs (0.20 μm in diameter; SD = 0.05, n = 29) of known composition [PC or PC:PE (1:1)] with PIP_2 micelles in deionized water at 36°C for 5 hours. Separation of LUVETs from micellar PIP_2 was performed by filtration of the mixture on a 0.7 cm by 10 cm Sepharose 2B column at room temperature, with a flow rate of buffer B of 30 ml/hour, and a fraction size of 0.4 ml. The fraction of PIP_2 incorporated in the LUVETs, about 50% of the total, was quantitated by liquid scintillation counting of the void volume. After filtration, the LUVETs remained 0.20 μm in diameter (SD = 0.02 μm, n = 27) and unilamellar. To be sure that the PIP_2 remained incorporated in these LUVETs, a sample was refiltered on Sepharose 2B 6 hours after preparation, by which time the PLC assays had been completed. As transverse diffusion of phospholipids in a bilayer (flip-flop) is slow, we considered in our calculations that all the PIP_2 was in the outer leaflet of the LUVETs. We could not rule out that some of the PIP_2 was incorporated into the inner leaflet of the vesicles or trapped as micelles inside the vesicles. However, the stoichiometry and affinity of profilin binding to these LUVETs as measured in the PLC assay was consistent with our other determinations if we assumed that all of the PIP_2 in these fused LUVETs was in the outer leaflet.
27. We are grateful to R. E. Pagano, A. Ting, D. M. Raben, A. S. Mildvan, and J. M. Baraban for helpful discussions and comments on the manuscript. Supported by NIH research grant GM-26338 to T.D.P. L.M.M. is supported by a graduate fellowship from NSF.

25 October 1989; accepted 25 January 1990

Binding of GAP to Activated PDGF Receptors

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The *ras* proto-oncogene products appear to relay intracellular signals via the Ras guanosine triphosphatase (GTPase) activator protein, GAP. In dog epithelial cells expressing human platelet-derived growth factor (PDGF) receptors, binding of PDGF caused approximately one-tenth of the total GAP molecules to complex with the receptor. Studies with mutant PDGF receptors showed that maximum association required both receptor kinase activity and phosphorylatable tyrosine residues at both the identified sites of receptor autophosphorylation.

THE PRODUCT OF THE *ras* PROTO-oncogene, Ras, is a guanine nucleotide binding protein (*1*). By analogy with *Saccharomyces cerevisiae* RAS gene products and the mammalian G proteins that couple membrane receptors to effector molecules such as adenylate cyclase, it is thought that the guanosine diphosphate (GDP) form of Ras is inactive, that the exchange of bound GDP for guanosine triphosphate (GTP) stimulates interaction between Ras and an effector molecule, and that GTP hydrolysis returns Ras to an inactive state (*2, 3*).

Ras is implicated in the control of cell growth. Oncogenic mutations in *ras* cause unregulated cell proliferation. In *Xenopus* oocytes, microinjection of oncogenic forms of Ras stimulate maturation (*1*). Microinjection of a monoclonal antibody to Ras into resting fibroblasts blocks mitogenic responsiveness to serum and to purified PDGF and epidermal growth factor (EGF) (*4*).

A guanosine triphosphatase (GTPase) activator protein known as GAP has properties of a mediator of signals generated by Ras (*5–7*). GAP was isolated on the basis of its ability to enhance the weak GTPase activity of normal Ras. Oncogenic forms of Ras are not sensitive to GAP, and persist as GTP complexes. GAP action on normal Ras converts it to a GDP complex. In this way, GAP may attenuate signaling by normal Ras-GTP. Several results suggest that GAP may itself be the effector through which Ras-GTP transmits a mitogenic signal to the cell. Mutagenesis of the GAP interaction domain on oncogenic forms of Ras blocks signaling (*6, 8*). In the *Xenopus* oocyte system, injection of a truncated form of Ras that has increased affinity for GAP is able to block some effects of oncogenically activated Ras, and excess GAP protein overcomes this

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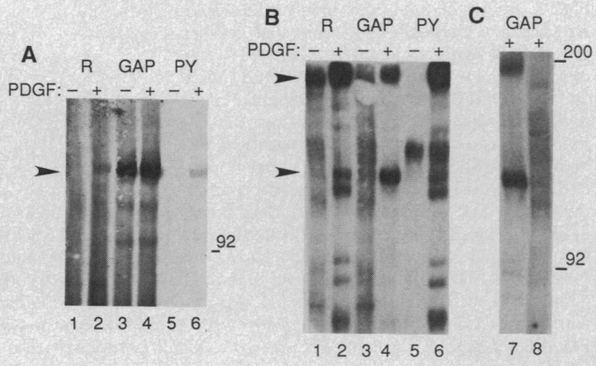


Fig. 1. GAP and the activated PDGFR coimmunoprecipitate. Quiescent cultures of dog kidney epithelial cells expressing approximately 10^5 human PDGFR β subunits per cell were treated for 5 min with (+) or without (−) the BB form of PDGF (40 ng/ml), then lysed and immunoprecipitated with either anti-PDGFR antibody PR7212 (R), anti-GAP antiserum (GAP), or anti-PY antibody 1G2 (PY) (*19*). Immunoprecipitates from 1.2×10^6 cells were incubated with [γ-^{32}P]ATP, resolved by electrophoresis and blotted (*20*). The immunoblot was probed with anti-GAP and the immunoreactive proteins were visualized with an alkaline phosphatase detection system. (**A**) Immunoreactive proteins. The arrowhead points to the prominent 124-kD GAP-immunoreactive species. (**B**) Autoradiogram of the blot in (A), showing the ^{32}P-labeled proteins. The top arrowhead points to the 180-kD PDGFR, and the lower arrowhead points to GAP. (**C**) Autoradiogram of in vitro phosphorylated proteins in anti-GAP immunoprecipitates prepared from PDGF-stimulated cells expressing the introduced PDGFR (lane 7) or an empty vector (lane 8). Molecular weight markers are in kilodaltons.

inhibition (*9*). In addition, Krev, a protein that blocks the transforming effects of oncogenic *ras* mutants on cells is very similar to Ras in the GAP-binding domain and may act by competing with Ras for binding to GAP (*10*).

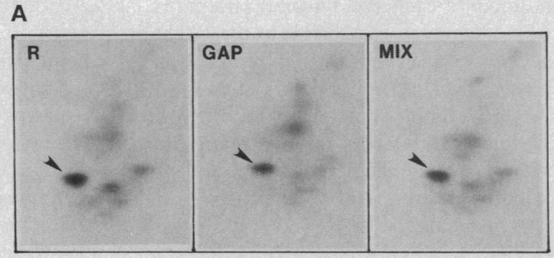
Stimulation of fibroblasts with EGF or PDGF, or transformation by oncogenes that encode tyrosine kinases, causes tyrosine phosphorylation of GAP (*11, 12*). In addition, GAP appears to be associated with two tyrosine-phosphorylated proteins, of 190 and 62 kD, in transformed and EGF-stimulated cells (*12*). Thus GAP activity may be regulated by tyrosine phosphorylation or association with specific cellular proteins.

There are two distinct PDGF receptor (PDGFR) genes, α and β, each encoding a transmembrane protein with an intracellular tyrosine kinase domain (*13*). Binding of the appropriate form of PDGF activates tyrosine phosphorylation (*14*) and ultimately triggers cell proliferation. Tyrosine phosphorylation of the β subunit of the human PDGFR triggers binding to cell proteins of 120, 84, and 72 kD, a phosphatidylinositol (PI) 3 kinase activity, and the serine kinase Raf (*15, 16*). We now report that activated PDGFRs also bind to GAP, suggesting that regulation of GAP function may participate in mediating PDGF's biological actions.

To test for GAP-PDGFR interactions, we used dog kidney epithelial cells expressing an introduced human β subunit of the PDGFR (*17*). Cells were incubated for 5 min with or without PDGF (*18*), lysed, and immunoprecipitated with antibodies to the PDGFR, GAP, or phosphotyrosine (PY) (*19*). Immunoprecipitated proteins were resolved by SDS–polyacrylamide gel electrophoresis, blotted, and probed with anti-GAP (*20*). As expected, the anti-GAP immunoprecipitates contained an immunoreactive 124-kD species (Fig. 1A, lanes 3 and 4). The quantity of immunoprecipitated GAP was not altered by PDGF treatment. A 124-kD protein reactive with anti-GAP was also present in anti-PY immunoprecipitates from PDGF-treated but not control cells (Fig. 1A, lanes 5 and 6). This observation is consistent with the tyrosine phosphorylation of GAP itself, or of GAP-associated proteins, in mitogen-stimulated cells (*12*). Anti-PDGFR immunoprecipitates, prepared from PDGF-treated but not control cells, also contained a 124-kD protein that reacted with anti-GAP (Fig. 1A, lanes 1 and 2), suggesting that GAP forms complexes with the activated PDGFR. A candidate GAP protein was also observed when PDGFRs were immunoprecipitated from PDGF-treated 3T3 cells (*22*).

We have previously reported that when anti-PDGFR immunoprecipitates, prepared from PDGF-treated cells, are incubated with [γ-^{32}P]adenosine triphosphate (ATP) in vitro, the 180-kD PDGFR and associated proteins of 120, 84, and 72 kD are labeled (*16*). In these reactions we have also noticed minor 145-, 140-, 124-, and 65-kD species (*22*). All of the associated proteins were more readily detected when immunoprecipitates were prepared from cells lysed under milder conditions and resolved by prolonged electrophoresis (Fig. 1B, lanes 1 and 2). Immunoblotting of ^{32}P-labeled reaction products showed that the 124-kD protein comigrated with GAP. Like the other PDGFR-associated proteins, the 124-kD band was only detected with PDGFRs prepared from PDGF-treated cells and was phosphorylated on tyrosine in vitro.

These results reveal that a GAP-PDGFR complex can be precipitated with anti-PDGFR antibodies, and suggest that GAP is a substrate for the PDGFR kinase. To test whether the putative complex could also be



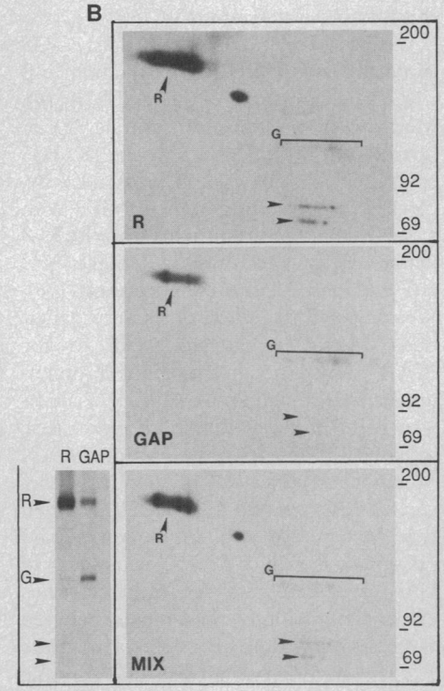


Fig. 2. Comparison of the radiolabeled phosphoproteins in GAP and PDGFR immunoprecipitates prepared from PDGF-treated cells. (**A**) Tryptic phosphopeptide maps of the 180-kD radiolabeled protein from PDGFR (R) and GAP (GAP) immunoprecipitates and a mixture of the two samples (MIX) (*24*). The arrowhead points to the phosphopeptide previously shown to contain Y^{751} (*16*). (**B**) GAP and PDGFR immunoprecipitates were prepared from PDGF-treated cells, labeled as described in Fig. 1 and resolved by two-dimensional gel electrophoresis (*20, 21*). The insert to the left of the bottom panel shows the same samples resolved on a one-dimensional SDS gel. The arrowhead labeled R points to the PDGFR, the bracket labeled G indicates the 124-kD GAP, and the two unlabeled arrowheads mark the 84- and 72-kD proteins previously detected in PDGFR immunoprecipitates from activated cells. Molecular weight markers are in kilodaltons.

precipitated with anti-GAP, we looked for PDGFR kinase activity in anti-GAP immunoprecipitates. Anti-GAP immunoprecipitates from PDGF-treated, but not control, cells contained a kinase activity that labeled 180-kD and 124-kD proteins that comigrated with the PDGFR and GAP, respectively (Fig. 1B, lanes 2 to 4). These bands were not detected in anti-GAP immunoprecipitates prepared from PDGF-treated cells that lack PDGFRs (Fig. 1C, lane 8). The 190-kD band previously found associated with GAP (*12*) appears not to be the PDGFR (*22*).

Further evidence that the 180-kD protein in anti-GAP immunoprecipitates was the PDGFR provided by two-dimensional gel electrophoresis and phosphopeptide mapping (Fig. 2). The two-dimensional gel mobilities and phosphopeptides of the 180-kD species were the same irrespective of whether anti-GAP or anti-PDGFR was used for immunoprecipitation. Also, the 124-kD phosphorylated protein that reacts with anti-GAP antibodies had the same two-dimensional mobility whether it was immunoprecipitated with anti-GAP or anti-PDGFR (Fig. 2B).

The quantity of GAP coprecipitating with PDGFRs was estimated by probing immunoblots of samples of total cell lysate with anti-GAP (Fig. 3A, lanes 1 to 3). An anti-PDGFR immunoprecipitate contained approximately 1.7 times as much GAP as the crude cell lysate from one-seventeenth as many cells (Fig. 3, lane 3 and 7). Therefore approximately 10% of the total soluble GAP was immunoprecipitable with anti-PDGFR. Since not all PDGFRs may be recovered, this may be an underestimate.

We have previously shown that PDGF stimulates phosphorylation of the human β PDGFR at tyrosines (Y) 751 and 857 (*16*). Mutation of Y^{751} to phenylalanine (F) or glycine (G), or mutation of lysine (K) 635 to arginine (R) to abolish kinase activity, blocked the association of the PDGFR with 120-, 84-, and 72-kD proteins and with PI 3 kinase activity. In contrast, mutation of Y^{875} to F did not decrease these associations (*16*). We compared the effects of the same mutations on the binding of GAP to the PDGFR, by probing anti-PDGFR immunoprecipitates with anti-GAP (Fig. 3, lanes 4 to 15). Receptors lacking intrinsic kinase activity (R^{635} mutation) did not bind GAP. Mutation of either Y^{751} or Y^{857} to F decreased GAP binding to about 20% of wild type. Mutation of both of these residues did not appear to reduce further the amount of GAP associated with the receptor. Thus efficient formation of the complex between GAP and the PDGFR requires that the receptor have kinase activity and tyrosine residues at both known phosphorylation sites.

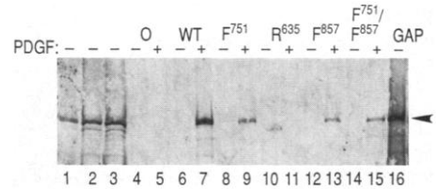


Fig. 3. Effects of mutations in the PDGFR on association with GAP. Dog epithelial cells expressing the various mutant PDGFRs (*17*) were treated with (+) or without (−) PDGF, lysed, and the PDGFR was immunoprecipitated as described in Fig. 1, except that anti-PDGFR PR7212 was covalently coupled to cyanogen bromide–activated Sepharose. Samples were normalized on the basis of cell number, the proteins resolved by SDS gel electrophoresis, transferred to Immobilon and probed with anti-GAP as described in Fig. 1. Lanes 1 to 3 are total cell lysates representing 2, 4, and 6% of the number of cells that were immunoprecipitated, respectively. Lane 16 is a GAP immunoprecipitate and marks the position of GAP (arrowhead). The amount of GAP was quantitated by densitometry; the signal in lanes 1 to 3 was linear with respect to the number of cells that was represented, allowing quantitation of the GAP associated with the PDGFR. Cells expressing an empty expression vector (O), wild-type PDGFR (WT), or PDGFR mutants F^{751}, R^{635}, F^{857}, and the double phosphorylation site mutant F^{751}/F^{857}.

The different requirements for the association of GAP relative to the 120-, 84-, and 72-kD proteins were confirmed by studying the in vitro phosphorylation of anti-PDGFR immunoprecipitates. Phosphorylation of the 120-, 84-, and 72-kD proteins was reduced much less than the phosphorylation of GAP by the F^{857} mutation, and phosphorylation of all the associated proteins was reduced severely by the F^{751} mutation (*22*).

In summary, our data suggest that GAP and the PDGFR form a complex after PDGF binding. The tyrosine phosphorylation of GAP in vitro further suggests that it may be a substrate for the PDGFR kinase. We also found that kinase-inactive receptor mutants did not associate with GAP, and that mutation of either or both of the receptor tyrosine autophosphorylation sites reduced the amount of associated GAP by 80%.

GAP joins the growing list of proteins that bind to the activated PDGFR. Binding of PI 3 kinase activity and of three proteins requires PDGF treatment, kinase activity, and Y^{751}, all prerequisites for phosphorylation of Y^{751} (*16*). Similarly, maximal binding of GAP requires PDGF treatment and intrinsic PDGFR kinase activity, suggesting that GAP binding is triggered not merely by a PDGF-induced allosteric change, but also by receptor-catalyzed tyrosine phosphorylation. However, in contrast to the binding of the other PDGFR-associated proteins, both known phosphorylation sites in the PDGFR seem to be equally important for the maximal binding of GAP. This may indicate that simultaneous phosphorylation of both residues, not just Y^{751}, is required for full binding. The distinct requirements for binding of GAP compared with certain other proteins suggests that GAP is not in a preformed complex with these other proteins.

Mutation of both known sites of tyrosine phosphorylation in the PDGFR did not reduce GAP binding to the same extent as inactivation of the PDGFR kinase. In living cells, PDGF stimulates detectable tyrosine phosphorylation of the F^{751}/F^{857} mutant, and this may permit low-level association of GAP. Alternatively, phosphorylation of GAP or a third protein may promote weak binding in the absence of either Y^{751} or Y^{857}. The level of GAP tyrosine phosphorylation is not known, but there is much less phosphotyrosine than phosphoserine in GAP from transformed cells (*12*). Elucidation of the requirements for GAP binding will require in vitro experiments and mutants in GAP as well as in the PDGFR.

Our findings and other reports that GAP is a substrate for tyrosine phosphorylation (*11, 12*) suggest that GAP may be a common node in signaling pathways used by Ras and tyrosine kinases. Binding of GAP could influence PDGFR activity, substrate specificity, or localization, and GAP functions could also be modified. One hypothesis is that association of cytosolic GAP with membrane-bound PDGFR may stabilize an active conformation of GAP, or bring it into proximity with other molecules lying downstream in a signaling pathway. Some GAP becomes membrane-associated after PDGF stimulation (*11*). Alternatively, binding to the PDGFR may decrease the ability of GAP to promote hydrolysis of GTP bound to Ras, increasing the effective concentration of the activated (Ras-GTP) form of GAP that relays the intracellular signal to proliferate. This latter hypothesis is consistent with the observation that microinjection of an antibody to Ras abolishes the proliferative response to PDGF and EGF (*4*). We have analyzed the guanine nucleotides bound to Ras in PDGF-treated cells, and detect no significant increase in the quantity of GTP. However, it is possible that only a small fraction of Ras is affected, and activation of a subpopulation of GAP molecules may be sufficient for mitogenesis. Consistent with either hypothesis, all PDGFR mutations that reduce GAP binding also reduce mitogenic responsiveness (*22*), although

these mutations have pleiotropic effects. In addition, we find that the mutant receptor that lacks both phosphorylation sites, but binds 20% of the GAP bound to wild-type PDGFRs, does not stimulate DNA synthesis in response to PDGF, suggesting that association of a small quantity of GAP is insufficient to trigger a biological response.

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17. Dog kidney epithelial TRMP cells [M. S. Turker *et al.*, *Cell Biol. Toxicol.* **4**, 211 (1988)] that lack endogenous PDGFR were infected with a retroviral expression vector containing a human β subunit PDGFR cDNA and a neomycin phosphotransferase gene, and resistant populations were selected in G^{418} (*16*). The virus stocks used in this study were high titer stocks from cloned packaging cell lines, and the resulting epithelial cell populations expressed approximately ten times as many receptors per cell relative to those described previously (*16*). Immunoprecipitation of [^{35}S]methionine-labeled cells with PR7212 anti-PDGFR monoclonal antibody showed that TRMP cells expressing wild-type and mutant receptors had equivalent numbers of receptors to within a 10% range (*22*). TRMP cells infected with an empty vector showed no mitogenic response to PDGF, whereas those expressing the wild-type PDGFR were responsive, showing up to 40% of the response elicited by 10% serum (*22*).
18. PDGF was the BB isoform, purified to homogeneity from yeast cell cultures expressing a recombinant DNA clone.
19. Anti-GAP antibody was raised to residues 171–448 of GAP (*12*). Anti-PDGFR monoclonal antibody PR7212 recognizes an extracellular epitope [C. E. Hart, R. A. Seifert, R. Ross, D. F. Bowen-Pope, *J. Biol. Chem.* **262**, 10780 (1987)]. Anti-PY antibody 1G2 was used to immunoaffinity purify PY-containing proteins as described [R. D. Huhn *et al.*, *J. Cell. Biochem.* **39**, 129 (1989)].
20. SDS polyacrylamide gels contained 7.5% acrylamide and 0.19% bis acrylamide. For two-dimensional electrophoresis, immunoprecipitates were first subjected to isoelectric focusing at *p*H 3.5 to 10, then resolved in the second dimension on a 7.5% SDS polyacrylamide gel, as described (*21*). Immunoblots on Immobilon transfer membrane (Millipore) were probed with anti-GAP antiserum (*19*) and developed with an avidin, biotin, alkaline phosphatase detection system (Vector Laboratories).
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23. Cells were lysed in EB (*21*) and immunoprecipitated as previously described (*16*). Immunoprecipitates were phosphorylated in vitro, as described (*16*).
24. Phosphoproteins were extracted, digested with trypsin, and the phosphopeptides were resolved in two dimensions (*p*H 8.9 electrophoresis and chromatography in buffer 1), as described (*16*).

25. We thank D. Bowen-Pope and R. A. Seifert for PDGF, PR7212, and Sepharose-coupled PR7212 and F. McCormick for the GAP cDNA used to prepare GAP antisera. Supported by grants from the NIH (CA-28151), National Cancer Institute of Canada and American Cancer Society postdoctoral fellowship PF-3292.

11 December 1989; accepted 26 January 1990

A New Member of the Leucine Zipper Class of Proteins That Binds to the HLA DRα Promoter

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Several mutants derived from transformed human B cell lines are defective in expressing major histocompatibility complex (MHC) class II genes. The failure to express a class II gene in at least one such mutant line has been mapped to the MHC class II X box, a conserved transcriptional element in the promoter region. A complementary DNA encoding a DNA-binding protein (human X box binding protein, hXBP-1) whose target is the human DRα X box and the 3′ flanking region has now been cloned. This complementary DNA encoded a protein with structural similarities to the c-*jun* proto-oncogene product, and its target sequence was closely related to the palindromic target sequence of c-*jun*. Mutation of the hXBP-1 DNA target sequence decreased DRα promoter activity in vivo. These studies suggest that the hXBP-1 protein acts as a transcription factor in B cells.

TRANSCRIPTIONAL CONTROL OF EUkaryotic gene expression is usually mediated by sequence-specific transcription factors that bind to DNA. A number of DNA-binding activities specific for sequences upstream of class II genes have been identified; several of these factors bind to the highly conserved X box and Y box motifs of human (*1, 2*) and murine (*3–5*) class II genes. Experiments with mice bearing Eα transgenes have shown a functional role of X and Y box sequences in class II transcription; deletion of X or Y resulted in greatly diminished Eα expression as well as abnormal transcriptional start sites (*3, 6*). Furthermore, transfected class II genes containing the X box motif are only minimally transcribed in several human class II negative mutant B cells (*7–12*). For two such mutant lines, the defects in transcription mapped to the X box, suggesting a defect in the activity of proteins that act through this motif (*10, 12*). To isolate the genes and proteins that influence transcription through binding to the X box motif, we used a method developed by Singh *et al.* (*13*). A λgt11 human B cell cDNA expression library was screened with a high-affinity labeled X box target sequence. The oligonucleotide we used as a probe was X(Aα)44, a 44-bp oligonucleotide containing the murine AαX box as well as the interspace sequence separating the X and Y boxes. We had shown earlier that nuclear extracts from human B lymphoblastoid cell lines contained binding proteins that recognize residues within this 44-bp motif (*4*). From 750,000 plaques examined, we identified a clone, λhXBP-1, that remained positive after three rounds of screening and was negative with a control probe from an upstream region of the Aα gene.

To characterize the binding site and structure of λhXBP-1, we tested lysates made from λhXBP-1 for binding activity by gel retardation analysis; various oligonucleotides corresponding to human class II se-

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