for 30 min at 4°C. The beads were washed three times with NETN and incubated with 700 μl of radiolabeled, in vitro–translated protein in NETN at 4°C for 1 hour. The beads were washed five times in NETN, and the proteins were eluted by boiling for 5 min in SDS-PAGE loading buffer [2% SDS, 10% glycerol, and 60 mM tris (pH 6.8)]. For peptide competition experiments, 2 μg of wild-type or mutant E7 peptide was included in each binding assay.

34. COS cells were grown on 100 mM tissue culture plates in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum (FCS). We transfected cells with 10 μg of the pcDNA1/NEO–Elf-1 plasmid containing the full-length human Elf-1 cDNA under the control of the cytomegalovirus promoter using lipofectin (BRL, Gaithersburg, MD) according to the manufacturer's instructions. Forty-eight hours after transfection, the cells were washed with phosphate-buffered saline and lysed by incubation for 15 min at 4°C in EBC buffer [50 mM tris (pH 8.0), 120 mM NaCl, 0.5% NP-40, 0.1 mM aprotinin, 1 mM leupeptin, and 0.1 mM phenylmethylsulfonyl fluoride]. Before their use in binding reactions and immunoprecipitations, the cell extracts were cleared by centrifugation in a microfuge at 14,000 rpm for 15 min at 4°C. For immunoprecipitations, 300 μl of COS cell extract or 400 μl of normal T cell extract was mixed with 700 μl of NETN and 10 μl of Elf-1 antiserum or preimmune serum and incubated for 1 hour at 4°C. Protein A–Sepharose (Pharmacia) was washed three times with 4% bovine serum albumin in NETN. Protein A–Sepharose (30 μl) was added to each immunoprecipitation reaction and incubated for 45 min at 4°C. The beads were washed five times with NETN, and the proteins were eluted by boiling in SDS-PAGE loading buffer. For the protein immunoblot experiments, Rb and Elf-1 proteins were resolved by electrophoresis in 7.5% SDS gels (*11, 12*). Protein immunoblots were probed with the Rb mAb G3-245 (Pharmingen, San Diego, CA) or Elf-1 antiserum produced by five sequential immunizations of a rabbit with purified recombinant Elf-1 protein. This antiserum immunoprecipitated Elf-1 but failed to immunoprecipitate other Ets family members, including Ets-1, Ets-2, GA binding protein α, and PU.1. It immunoprecipitates a single major protein of 97 kD from normal human T cells and from Jurkat cells, which corresponds in size to that of in vitro–translated Elf-1. It does not directly immunoprecipitate Rb. The second antibody was commercially available horseradish peroxidase–coupled goat antibody to mouse immunoglobulin (Ig) or goat antibody to rabbit Ig (BRL).
35. Normal human T cells were isolated by density gradient centrifugation from buffy coats obtained from normal volunteers as described (*31*). All preparations used in these studies contained more than 95% lymphocytes as assessed by staining with Wright's stain. Human T cells were cultured at a density of $2 \times 10^6$ cells/ml in either RPMI + 10% FCS or activated by culturing in RPMI + 10% FCS + PMA (10 ng/ml) + ionomycin (0.5 μg/ml) for 24 to 48 hours before harvesting. T cell extracts were prepared in EBC buffer as described above ($400 \times 10^6$ cells per 0.5 ml of EBC buffer).
36. Electrophoretic mobility-shift assays (EMSAs) were carried out as described (*5*) with $^{32}$P-labeled double-stranded synthetic oligonucleotides corresponding to the wild-type (GATCGTCACCATTAATCATTTCCTCTAACTGT) or mutant (GATCGTCACCATTAATCATTTGGCATAACTGT) Elf-1 binding sites from the GM-CSF promoter.
37. Human Jurkat T cell tumor cells were cultured in RPMI + 10% FCS and transfected with DEAE-dextran as described previously (*5*). All transfections contained 4 μg of reporter plasmid and 1 μg of the pRSVβgal reference plasmid. Cells were cultured for 36 hours after transfection and then, when indicated, activated by treatment with PMA (40 ng/ml) + ionomycin (1.4 μg/ml) for 8 to 10 hours at 37°C. Cells were harvested and extracts assayed for CAT and β-galactosidase activity as described (*5*). All transfections were repeated at least three times.
38. We thank D. Livingston, J. Lowe, M. Parmacek, and G. Nabel for helpful discussions and P. A. Hamel for generously providing the pECE-Δp34-HA plasmid. C. McNicholas-Serwecinski provided expert secretarial assistance and B. Burck provided help with the preparation of illustrations. Supported in part by NIH grant RO1 AI29673-01 (J.M.L.).

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# Inactivation of the Type II Receptor Reveals Two Receptor Pathways for the Diverse TGF-β Activities

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**Transforming growth factor–β (TGF-β) is a multifunctional protein that regulates cell proliferation and differentiation and extracellular matrix production. Although two receptor types, the type I and type II receptors, have been implicated in TGF-β–induced signaling, it is unclear how the many activities of TGF-β are mediated through these receptors. With the use of cells overexpressing truncated type II receptors as dominant negative mutants to selectively block type II receptor signaling, the existence of two receptor pathways was shown. The type II receptors, possibly in conjunction with type I receptors, mediate the induction of growth inhibition and hypophosphorylation of the retinoblastoma gene product pRB. The type I receptors are responsible for effects on extracellular matrix, such as the induction of fibronectin and plasminogen activator inhibitor I, and for increased JunB expression. Selective inactivation of the type II receptors alters the TGF-β response in a similar manner to the functional inactivation of pRB, suggesting a role for pRB in the type II, but not the type I, receptor pathway.**

TGF-β is a secreted protein that inhibits proliferation of many cell types, regulates the expression of extracellular matrix proteins and their integrin receptors, and is considered an important physiological regulator of cell proliferation and differentiation (*1, 2*). Cross-linking experiments have revealed that most cells have three types of TGF-β receptors at the cell surface. The type III receptor (or betaglycan) is a proteoglycan with a short cytoplasmic domain (*3, 4*) and is not likely to mediate any of the known biological activities of TGF-β (*5–7*). The type II receptor has recently been cloned, and its sequence indicates that it is a serine-threonine kinase (*8*). Both type I and type II receptors have been implicated in mediating the biological activities of TGF-β (*5–7, 9*). Complementation studies with mutant mink lung epithelial cell lines (Mv1Lu) resistant to growth suppression by TGF-β indicated that the type I receptors or both type I and type II receptors are necessary for all tested biological responses to TGF-β (*5–7*). In complementation analyses using different cell hybrids, increased type II receptor expression was associated with growth suppression but not induction of several matrix proteins (*9*). However, the concomitant increase in type I receptor levels in this study (*9*) and the possible interaction between these two receptor types (*7, 10*) complicates the interpretation of these data. In addition, the growth inhibitory effect of TGF-β can be abolished by inactivation of pRB without affecting the ability of TGF-β to induce an increased gene expression, suggesting that pRB is not involved in a defined set of responses (*11*). So far, it has remained unknown whether these different activities of TGF-β are mediated through a single receptor complex with divergent signaling pathways or by separate receptor-associated pathways.

To determine the specific roles of the type II and type I receptors, one could overexpress the type II receptors or abolish the function of the type II receptors and then evaluate the resulting changes in the spectrum of biological activities of TGF-β. Our first approach was to overexpress the type II receptor in transfected cell lines. Because very few cell lines lack endogenous TGF-β receptors (*1, 9, 12*), and other components of the TGF-β receptor signaling pathways could possibly be defective in these cells, we overexpressed type II receptors in cells that contain a low level of TGF-β receptors and are sensitive to TGF-β, such as the Mv1Lu cells (*5*). However, transient transfection assays with expression vectors for the human type II receptor (*8*) in QT6 and 293 cells resulted not only in increased cell surface expression of type II receptors but also in increased availability of type I receptors (Fig. 1A). This increase of type I receptors may be the result of the suggested interaction between both TGF-β receptor types (*6, 7, 10*). Thus, even

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though transfected Mv1Lu cells overexpressing the type II receptors show an increased antiproliferative effect of TGF-β (*13*), these experiments do not permit an unambiguous assignment of the function to the type II receptor.

We therefore decided to down-regulate the type II receptor by using dominant negative mutants (*14*) that do not affect the type I receptors. Deletions of the cytoplasmic domains of several tyrosine kinase receptors, known to dimerize during ligand-induced activation (*15–17*), and most recently of a *Xenopus* receptor for activin, a member of the TGF-β family (*18*), have been shown to act as dominant negative mutations. We thus constructed an expression vector for a truncated type II receptor containing the complete extracellular and transmembrane domains but lacking most of the cytoplasmic domain, including the kinase domain (*8*). An eight–amino acid epitope tag was added to allow immunoprecipitation with a specific monoclonal antibody. This expression plasmid was first tested in transient transfection assays in QT6 and COS-1 cells because of their high transfection efficiencies. Chemical cross-linking with [$^{125}$I]TGF-β showed that untransfected QT6 cells expressed the three common TGF-β receptor types at their surface (Fig. 1B, lane 1). The transfected cells contained an extra binding component of lower molecular weight corresponding to the truncated receptor (Fig. 1B, lane 3), which could be specifically immunoprecipitated with an antibody directed against the COOH-terminal epitope tag (Fig. 1B, lane 4). Thus, the truncated form is exposed at the cell surface and retains the ability to bind TGF-β. Furthermore, the expression of the truncated receptors markedly decreased the endogenous type II receptor level but did not affect the level of cross-linked type I receptors (Fig. 1B, lane 3). This suggests that there may be a specific interaction between the truncated and the wild-type form of the type II receptor, possibly resulting in degradation or impaired transport to the cell surface. Because our transfection efficiency in QT6 cells is as high as 80% (*13*), the cross-linked endogenous type II receptor in the pool of transfected cells is presumably due to the remaining 20% of the cells. In a parallel experiment (Fig. 1B, lane 2), transfection with a similar expression plasmid encoding a truncated, structurally related serine-threonine kinase receptor with different ligand specificity (*13*), did not affect the expression and availability of the endogenous type II TGF-β receptors. Our findings were identical in both the QT6 cells (Fig. 1B) and the COS-1 cells (*13*). Taken together, our results indicate that the truncated type II TGF-β receptors act effectively and specifically on the type II receptors but do not alter the availability of the type I receptor.

Transfected cells that stably overexpress the truncated type II receptor were generated to evaluate resulting alterations in the biological activities of TGF-β. Mv1Lu cells were used as the target cell line because of their high sensitivity to the various biological effects of TGF-β (*1*, *2*). Because of the intrinsic competitive nature of dominant negative mutants, the expression level of the truncated receptor needs to greatly exceed that of the endogenous receptor. Among the transfected Mv1Lu cell clones, we obtained two clones with high levels of truncated receptor mRNA (*13*) and truncated cell surface receptor (Fig. 2, A and B) (*13*). As a result of the extremely high level of truncated receptors, the expression of endogenous type I and type II receptors at the surface could not be determined by cross-linking assays. However, transfected Mv1Lu cells producing a much lower level of the truncated receptor had a decreased level of endogenous type II receptor and an unaltered type I receptor level, as expected from our results in the transient transfection assays (Fig. 2C).

Exposure to 100 pM TGF-β1 inhibited DNA synthesis almost completely in the parent cells but hardly affected the DNA synthesis in the cells overexpressing the truncated receptor. Even at 1000 pM TGF-β1, DNA synthesis was only minimally suppressed in the transfected cells (Fig. 3A). Similar results were obtained when the cells were treated with TGF-β2 (*13*). The response of both transfected cell clones was equivalent. Accordingly, the proliferation of the cells overexpressing the truncated receptor was barely affected by 100 pM TGF-β1, whereas the growth of the parent

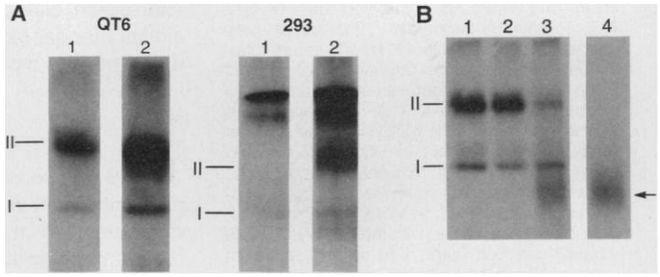


**Fig. 1.** Cross-linking of [$^{125}$I]TGF-β1 to the surface of mock-transfected cells or cells transfected with an expression vector for either the complete type II receptor (**A**) or the truncated type II receptor (**B**). In (A), QT6 or 293 cells were transfected with either salmon sperm DNA (lanes 1) or the pRK5 (*26*)-based expression vector containing the human type II TGF-β receptor cDNA (lanes 2) (*27*). The type I and type II TGF-β receptors are indicated by Roman numerals. The type III receptor is at the top of the gel in the QT-6 cross-linking pattern and corresponds to the darkest band above the type II receptor in the 293 cell pattern. In (B), QT6 cells were transfected with salmon sperm DNA (lane 1), the pRK5 expression plasmid containing the truncated human type II TGF-β receptor (lane 3) (*25*), or the same expression vector containing a truncated form of a structurally related serine-threonine kinase receptor with different ligand specificity (lane 2). In lane 4, the cells were transfected and cross-linked as in lane 3, but an immunoprecipitation (*28*) of the cell lysate was performed with a monoclonal antibody against the COOH-terminal epitope tag (*22*) in the truncated type II receptor. Arrow indicates band corresponding to the truncated receptor.

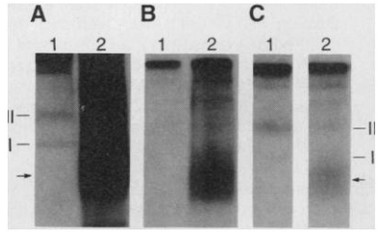


**Fig. 2.** Expression of the truncated type II TGF-β receptors in stably transfected Mv1Lu cells. Cross-linking of [$^{125}$I]TGF-β to the cell surface receptors was done with the parental Mv1Lu cells (**A** and **B**, lanes 1) and the stably transfected Mv1LuDN1 cells (A and B, lanes 2). The cross-linked complexes were separated by denaturing and reducing polyacrylamide (7.5%) gel electrophoresis and exposed for autoradiography for 24 hours (A) or 8 hours (B). In (**C**), the cross-linking pattern for cells expressing a low level of the truncated type II receptors (Mv1LuDN0 cells) was compared in lane 2 with the parent Mv1Lu cells (lane 1). The Eco RI–Xba I fragment containing the truncated type II receptor cDNA (*25*) was blunted with DNA polymerase I (Klenow) and ligated into the Eco RV site of the expression vector pCDNA-1neo (Invitrogen), a cytomegalovirus promoter–based expression plasmid. The resulting plasmid was transfected into the Mv1Lu cells by lipofection according to the procedure recommended by Boehringer Mannheim Corp. The transfected cells were incubated in culture medium containing G418 (600 μg/ml). After 14 to 21 days, G418-resistant clones were selected, and the expression of truncated type II receptor mRNA was examined by Northern (RNA) blot analysis. Two clones (Mv1LuDN1 and Mv1LuDN2) expressing a high level of truncated receptor mRNA and one clone (Mv1LuDN0) expressing a lower level of truncated receptor mRNA were chosen for cross-linking analysis according to the procedure described in Fig. 1. Mv1LuDN2 cells showed a similar cross-linking pattern as Mv1LuDN1 cells (*13*). Arrows indicate bands corresponding to the truncated receptor.

cells was almost totally inhibited (*13*). These results thus strongly suggest that the type II receptor plays a central role in the antiproliferative effect of TGF-β.

Treatment of Mv1Lu cells with TGF-β has been shown to prevent phosphorylation of pRB and arrests the cells in late prereplicative phase of the cell cycle ($G_1$) (*19*). In the exponentially growing Mv1Lu cells, most of the pRB protein was in the hyperphosphorylated state, whereas treatment with TGF-β led to an accumulation of underphosphorylated pRB (Fig. 3B). In contrast, this TGF-β–induced inhibition of pRB phosphorylation was not observed in cells overexpressing the truncated type II receptor (Fig. 3B).

We then examined whether the induction of plasminogen activator inhibitor type 1 (PAI-1) and fibronectin synthesis by TGF-β was affected in the transfected cells. In contrast to the inhibition of cell proliferation, both the parental and transfected cells responded equally to TGF-β with an increased production of both PAI-1 and fibronectin (Fig. 4A). The synthesis of PAI-1 and fibronectin in the transfected cells was strongly increased at TGF-β concentrations as low as 10 pM, even though a 100-fold higher concentration had almost no effect on the proliferation of these cells (Fig. 3A). Thus, our results suggest that the type II receptor is not required for stimulation of the synthesis of these two extracellular matrix proteins. Finally, TGF-β also induced a similar increase in JunB mRNA levels in both the parent and transfected cell lines, indicating that this response, too, was not dependent on functional type II receptors (Fig. 4B).

Overexpression of the truncated type II receptor specifically inhibits the function of the endogenous type II receptors at the cell surface and abolishes specific cellular responses, indicating that the truncated receptor functions as dominant negative mutant (*14*). By analogy with other receptor kinases (*15–17*), including the related activin receptor (*18*), the simplest model is that truncated and wild-type receptors form heterodimers and in this way abrogate a functional response. The reduced level of endogenous type II receptor may then be due to an accelerated degradation of the heterodimers of the truncated and wild-type type II receptors or by an impaired transport of the wild-type receptor to the cell surface. A physical interaction between the type II and type I receptors may also exist, resulting in a heterodimeric TGF-β receptor (*10*). If so, the truncated type II receptor could form a heterodimer with the type I receptor and in this way exert its specific dominant negative effect by preventing an interaction of the endogenous, functional type II receptor with the type I receptor. On the other hand, one would then have to assume that this would not adversely affect the stability and function of the type I receptor because overexpression of the truncated receptor did not affect the availability of the type I receptor at the cell surface and a distinct set of responses of TGF-β. Thus, our results indicate that the truncated type II receptor functions as a specific dominant negative mutant of the type II, but not the type I, receptor.

Our results indicate that two distinct receptor-associated signaling pathways exist, each mediating a separate set of TGF-β activities. The type II receptor is required for the inhibition of pRB phosphorylation and for the antiproliferative effect, but not for the stimulation of fibronectin and PAI-1 synthesis and for the induction of JunB mRNA, all of which therefore have to be assigned to the type I receptor. Previous analyses using mutants derived from the same Mv1Lu cell line led to the conclusion that either the type I receptor or both type I and type II receptors are necessary for all tested responses to TGF-β (*5–7*). The discrepancy between our current findings and these previous conclusions may, at least in part, be due to the lack of mutant Mv1Lu cell lines that had normal type I receptors but no type II receptors. Whereas our results indicate that the TGF-β–induced inhibition of cell proliferation and pRB phosphorylation are mediated through the type II receptor, we cannot determine whether

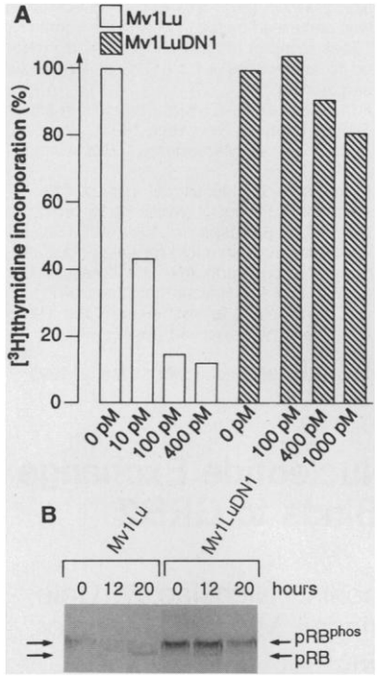


**Fig. 3.** Abolition of TGF-β–induced antiproliferative effect and inhibition of pRB phosphorylation in cells overexpressing truncated type II receptors. (**A**) Comparison of TGF-β–induced inhibition of DNA synthesis in parental Mv1Lu cells and the transfected Mv1LuDN1 cells overexpressing the truncated type II TGF-β receptor. Cells were plated at a density of $5 \times 10^4$ per square centimeter in the absence or presence of various concentrations of TGF-β1 as indicated. After a 36-hour incubation, [$^3$H]thymidine (1 μCi/ml) was added to each culture, the cultures were incubated for 3 hours, and the incorporation of [$^3$H]thymidine into acid-insoluble material was measured (*29*). Incorporated counts per minute were normalized to $10^5$ cells, and the percentage decrease in the normalized incorporation relative to cultures that received no TGF-β is presented. Mv1LuDN2 cells, another clone overexpressing the truncated type II receptor, provided results similar to those of Mv1LuDN1 cells (*13*). In addition, exposure of the cells to TGF-β2 gave essentially the same results. (**B**) TGF-β effect on pRB phosphorylation in Mv1Lu and Mv1LuDN1 cells. Exponentially growing cells were incubated with or without 100 pM TGF-β for the indicated times, and the state of pRB phosphorylation (pRB$^{phos}$) was determined by immunoblot analysis as described (*19*).

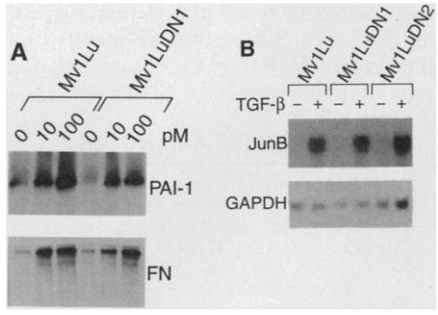


**Fig. 4.** Stimulation of PAI-1 and fibronectin synthesis (**A**) and JunB mRNA expression (**B**) in response to TGF-β1. (**A**) Subconfluent cultures of parental Mv1Lu cells and the transfected Mv1LuDN1 cells overexpressing the truncated type II receptor were treated with TGF-β1 for 12 hours (for fibronectin analysis) or for 2 hours (for PAI-1 analysis) and labeled in the serum-free, Cys,Met-free medium containing 25 μCi/ml of [$^{35}$S]Cys and [$^{35}$S]Met in the absence or presence of the indicated concentrations of TGF-β1 for 2 hours. Fibronectin secreted into the labeled medium was affinity-purified with gelatin-Sepharose beads and analyzed by denaturing gel electrophoresis as described (*30*). For analysis of PAI-1, extracellular matrix proteins were harvested as described (*11*) and separated under reducing conditions by denaturing polyacrylamide (10%) electrophoresis. PAI-1 was identified as a TGF-β–inducible band of 45 kD (*11*). In (B), exponentially growing cultures of parental Mv1Lu cells and transfected Mv1LuDN1 and Mv1LuDN2 cells were treated with or without 100 pM TGF-β1 for 1 hour. Total RNA was prepared and was assayed for JunB and glyceraldehydephosphate dehydrogenase (GAPDH) mRNA levels by northern blot analysis (*28*).

the type I receptor is required for this process. However, the existence of TGF-β–unresponsive Mv1Lu cell mutants that lack type I receptors yet have normal levels of type II receptors (5) suggests that type I receptors may be needed to mediate these activities of TGF-β through the type II receptor.

Our findings also indicate that the signaling through the type I receptor does not require a functional type II receptor to mediate autonomously its distinct set of TGF-β–induced activities. This is consistent with the fact that 293 cells, which lack detectable levels of type II receptors but have type I receptors (Fig. 1A), are not responsive to the antiproliferative activity of TGF-β yet display a TGF-β–induced synthesis of fibronectin (*20*). These data further support our conclusion that the type II receptor is required for and mediates the antiproliferative effect of TGF-β, whereas the induction of fibronectin synthesis is mediated by the type I receptor. Moreover, the wide variability and lack of correlation between the cell surface levels of the type I and type II receptors suggest that besides possible heterodimers, there may be type I and type II receptors that do not physically interact with each other and that perhaps could function as homodimers.

Finally, the selective functional abolition of the type II receptors results in alterations in the complex response to TGF-β similar to the functional inactivation of pRB by viral transforming proteins such as the SV40 large T antigen, that is, a specific abrogation of the antiproliferative effect of TGF-β (*11, 21*) without affecting the induction of expression of several genes by TGF-β (*11*). This very similar phenotype thus indicates that the role of pRB in the response to TGF-β is specific for and restricted to the signaling pathway associated with the type II receptor. Accordingly, our transfected cells lacking functional type II receptors did not show TGF-β–induced inhibition of pRB phosphorylation, suggesting that type II receptors are required for the effect of TGF-β on the phosphorylation state of pRB.

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25. To generate the truncated type II receptor, we made an Eco RI–Hpa I fragment corresponding to nucleotides −7 through 573 of the type II receptor by polymerase chain reaction (PCR), followed by cleavage at the unique Hpa I site located immediately 3′ of the transmembrane domain sequence. A double-stranded oligonucleotide adaptor for the sequence encoding the epitope tag, FLAG (*22*), was ligated to the 3′ end of the truncated type II cDNA, and the resulting fragment was inserted into the Eco RI and Xba I sites of the expression vector pRK5, thus generating the expression plasmid for the truncated type II receptor. DNA was transfected into the QT6 cells and 293 cells by calcium phosphate precipitation (*23*). Cross-linking analysis was performed as described (*3, 24*), and the cross-linked proteins were separated by denaturing and reducing polyacrylamide (7 5%) gel electrophoresis and subsequent autoradiography.
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27. The expression vector for the complete human type II TGF-β receptor was made by insertion of an Eco RI fragment containing the entire cDNA coding sequence into the Eco RI opened pRK5, a human cytomegalovirus promoter–based expression plasmid (*26*). This Eco RI cDNA fragment was generated by the PCR with the human type II TGF-β receptor (*8*) as template and corresponded to nucleotides −7 to 1703 of the published sequence (*8*).
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# Human Sos1: A Guanine Nucleotide Exchange Factor for Ras That Binds to GRB2


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A human complementary DNA was isolated that encodes a widely expressed protein, hSos1, that is closely related to Sos, the product of the *Drosophila* son of sevenless gene. The hSos1 protein contains a region of significant sequence similarity to CDC25, a guanine nucleotide exchange factor for Ras from yeast. A fragment of hSos1 encoding the CDC25-related domain complemented loss of CDC25 function in yeast. This hSos1 domain specifically stimulated guanine nucleotide exchange on mammalian Ras proteins in vitro. Mammalian cells overexpressing full-length hSos1 had increased guanine nucleotide exchange activity. Thus hSos1 is a guanine nucleotide exchange factor for Ras. The hSos1 interacted with growth factor receptor–bound protein 2 (GRB2) in vivo and in vitro. This interaction was mediated by the carboxyl-terminal domain of hSos1 and the Src homology 3 (SH3) domains of GRB2. These results suggest that the coupling of receptor tyrosine kinases to Ras signaling is mediated by a molecular complex consisting of GRB2 and hSos1.


*Ras* genes encode membrane-bound guanine nucleotide binding proteins that function in the transduction of signals that control cell growth and differentiation. Binding of guanosine triphosphate (GTP) activates Ras proteins, and subsequent hydrolysis of the bound GTP to guanosine diphosphate (GDP) inactivates signaling by these proteins. GTP binding can be catalyzed by guanine nucleotide exchange factors for Ras and GTP hydrolysis can be accelerated by GTPase activating proteins (GAPs) (*1*). The first exchange factor for Ras to be identified was the CDC25 gene


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