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Type I Receptors Specify Growth-Inhibitory and Transcriptional Responses to Transforming Growth Factor β and Activin

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Transforming growth factor β (TGF-β) and activin bind to receptor complexes that contain two distantly related transmembrane serine/threonine kinases known as receptor types I and II. The type II receptors determine ligand binding specificity, and each interacts with a distinct repertoire of type I receptors. Here we identify a new type I receptor for activin, ActR-IB, whose kinase domain is nearly identical to that of the recently cloned TGF-β type I receptor, TβR-I. ActR-IB has the structural and binding properties of a type I receptor: it binds activin only in the presence of an activin type II receptor and forms a heteromeric noncovalent complex with activin type II receptors. In Mv1Lu lung epithelial cells, ActR-IB and TβR-I signal a common set of growth-inhibitory and transcriptional responses in association with their corresponding ligands and type II receptors. The transcriptional responses include elevated expression of fibronectin and plasminogen activator inhibitor 1. Although TβR-I and ActR-IB are nearly identical in their kinase domains (90% amino acid sequence identity), their corresponding type II receptor kinase domains are very different from each other (42% amino acid sequence identity). Therefore, signaling of a specific set of responses by TGF-β and activin correlates with the presence of similar type I kinases in their complex. Indeed, other TGF-β and activin type I receptors (TSR-I and ActR-I) whose kinase domains significantly diverge from those of TβR-I and ActR-IB do not substitute as mediators of these growth-inhibitory and extracellular matrix transcriptional responses. Hence, we conclude that the type I receptor subunits are primary specifiers of signals sent by TGF-β and activin receptor complexes.

The related cytokines transforming growth factor β (TGF-β) and activin have caught the attention of biologists for different reasons. TGF-β can inhibit or stimulate cell proliferation, promote extracellular matrix formation, regulate cell differentiation, and affect many other functions in mammalian cells of virtually every lineage (37, 47). The activins are also multifunctional, acting as regulators of endocrine cells from the reproductive system (34, 58, 59), promoters of erythroid differentiation (13), and inducers of axial mesoderm and anterior structures in vertebrates (18, 50–52, 56, 60). Receptors that bind TGF-β and activin have been cloned recently. However, what determines the multifunctional nature of these cytokines and specifies their diverse responses remains unknown.

TGF-β and activin exist in various isoforms generated by homodimeric and heterodimeric combinations of at least three (TGF-β1, -β2, and -β3) or two (activin-βA and -βB) closely related gene products (37, 47, 58). TGF-β, activins, and a third group of factors in this family, the bone morphogenetic proteins (35, 48), each interact with pairs of membrane proteins known as receptor types I and II (32, 38). One TGF-β type II receptor, TβR-II (33), and two activin type II receptors, ActR-II and ActR-IIB (3, 39, 41), have been cloned from mammals and other vertebrates. Only one bone morphogenetic protein type II receptor, the *daf-4* gene product from *Caenorhabditis elegans*, has been identified to date (12). These receptors are transmembrane proteins that contain a short, cysteine-rich extracellular region and a cytoplasmic region dominated by a serine/threonine kinase domain. The extracellular region of the *Drosophila* activin type II receptor, Atr-II, shows little sequence similarity to the vertebrate activin receptors, except for the spacing of cysteines which is strictly conserved and probably critical in determining ligand binding specificity (10). This region shows almost no sequence similarity to the extracellular regions of TβR-II and daf-4, and the kinase domains of these receptors are also widely divergent.

The properties of cell mutants that are deficient in TGF-β receptor I or II indicate that these two molecules are interdependent components of a signaling receptor complex (5, 24, 30, 31, 62). The type I receptor requires receptor II in order to bind ligand (62). The type II receptor expressed alone can bind ligand, but it requires association with a type I receptor in order to signal. These conclusions have been confirmed with the recent cloning of TGF-β and activin type I receptors from mammals and *Drosophila melanogaster* (2, 4, 11, 15, 57). Surprisingly, these receptors are also transmembrane serine/threonine kinases and were in fact cloned in searches for type II receptor related genes.

The type I receptors represent a distinct subfamily with kinase domains that are more similar to each other than to the kinase domains of type II receptors. All type I receptors found to date show similar patterns of extracellular cysteines. Known type I receptors include TβR-I, which binds TGF-β in concert with TβR-II (4, 15), ActR-I (2), also known as SKR1 (42) or Tsk7L (11), which binds activin in concert with ActR-II or IIB (2, 11), the dual type I receptor TSR-I, which binds TGF-β in concert with TβR-II and activin in concert with ActR-II (2), and the *Drosophila* activin type I receptor Atr-I, which interacts with Atr-II (64). Additional family members have been cloned (55), but their ligands remain to be identified.

The present study identifies a new human activin type I receptor, ActR-IB, whose kinase domain is nearly identical to

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that of TβR-I. However, the ectodomains of these receptors show low sequence similarity and lack of affinity for each other's ligand. It is becoming clear that TGF-β and activin each have at their disposal a small repertoire of heteromeric receptor combinations. Such diversity may underlie the diversity of responses induced by these factors. The functional roles of these receptors are largely unknown, and important responses such as growth inhibition have not been assigned to any specific receptor combination.

A more fundamental question, what component of the heteromeric receptor complex confers signaling specificity, remains open as well. Here, we address these questions by analyzing the signaling activity of TβR-I and ActR-IB. Acting in concert with their corresponding type II receptors, which are divergent from each other, TβR-I and ActR-IB mediate similar sets of responses to their respective ligands. The evidence argues that the type I receptors are primary specifiers of signals sent by the receptor complexes in which they participate. The responses specified by TβR-I and ActR-IB include cell cycle arrest and synthesis of extracellular matrix proteins, two well-documented TGF-β responses that now appear also as activin responses.

MATERIALS AND METHODS

Isolation of ActR-IB cDNA. To obtain a full-length ActR-IB cDNA, a DNA fragment encoding part of the kinase domain (nucleotide 706 to 1377) was prepared by PCR based on the sequence of SKR2 (65). cDNA was generated from random-primed total RNA prepared from A549 and HepG2 cells as described previously (3, 63). The cDNA was amplified by PCR using primer A (5′-AAAATATTCTCTTCTCGTGAAGAA-3′) and primer B (5′-CCAGTTGGGGATGTTGGGA-3′) for 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s on a Perkin-Elmer Thermal Cycler 9600. The PCR-derived probe was used to screen a human kidney λgt11 cDNA library (Clontech) and yielded multiple isolates, each partially encoding a cDNA for an alternate form of SKR2, namely, ActR-IB. A full-length ActR-IB cDNA was constructed from two fragments, each encoding half of the cDNA, generated by PCR from randomly primed total RNA.

Expression vectors. ActR-IB expression vectors were constructed in pCMV5 (1) for transient assays and in pMEP4 (Invitrogen) for the generation of stable transfectants, using convenient restriction sites. A PCR-based mutagenesis strategy was used to generate the K232R mutant in TβR-I, and the mutation was verified by sequencing the insert. TβR-I and TβR-I(K232R) were tagged in the C terminus with the influenza virus hemagglutinin (HA) epitope (44). The HA-tagged constructs were subcloned into pCMV5, using convenient restriction sites. The construction of pCMV5-ActR-II, pCMV5-ActR-II-His, pCMV5-TβR-II, pCMV5-TβR-II-HA, pCMV5-TβR-II(K277R)-HA, pCMV5-TSR-I, and pCMV5-ActR-I has been described previously (2, 3, 62). For stable transfectants, the ActR-I, TSR-I, and TβR-I cDNAs were subcloned into pMEP4, using convenient sites in the polylinker region of the vector.

Cell lines and transfections. The Mv1Lu mink lung cell line (CCL-64; American Tissue Culture Collection) and the mutant cell lines were maintained in minimal essential medium (MEM; Gibco-BRL) supplemented with 10% fetal bovine serum (FBS) and nonessential amino acids. COS-1 cells were maintained in Dulbecco's modified Eagle medium (Gibco-BRL) supplemented with 10% FBS.

Transient transfection of COS-1 cells with the appropriate receptor vectors was done by the DEAE-dextran method (49), and cells were assayed 48 to 72 h after transfection. Transient transfections of parental and mutant Mv1Lu cell lines with the TGF-β-inducible reporter construct p3TP-lux and appropriate receptor vectors were done as previously described (62). Cells were plated into six-well plates at 75,000 to 120,000 cells per well and grown for 2 days until they were about 50% confluent. They were then washed twice with MEM containing amphotericin B (Fungizone) and gentamicin (MEM-f/g) and incubated for 2 min in 1 ml of MEM-f/g containing 100 μM chloroquine. To this medium was added 340 μl of DNA-dextran mix (170 μl of phosphate-buffered saline [PBS], 3 μg of p3TP-lux DNA, 1 μg of receptor vector [or pCMV5] DNA, 170 μl of a solution containing 1 mg of dextran per ml and 0.45 mg of NaCl per ml). Cultures were incubated at 37°C for 3.5 h and then shocked for no longer than 2 min with 10% dimethyl sulfoxide in PBS. Cell were then washed in MEM-f/g containing 10% FBS and incubated overnight in this medium.

Stable R-1B cell transfectants expressing the appropriate receptors were generated by transfection with Lipofectin (Gibco-BRL) as previously described (62).

Receptor assays. TGF-β1 from porcine platelets (R&D Systems) was labeled with ^{125}I as previously described (8). Activin-A (gifts from Y. Eto, Ajinomoto Co., and Jennie Mather, Genentech Inc.) was labeled with ^{125}I as previously described (3). Cell monolayers were incubated for 3 h at 4°C with 250 pM ^{125}I-labeled ligands unless otherwise indicated. Receptors were cross-linked to bound ligands with disuccinimidyl suberate (Pierce Chemical Co.) and solubilized in the presence of Triton X-100 as previously described (36). Cell extracts were clarified by centrifugation and then subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and autoradiography. Immunoprecipitation with antibodies against ActR-II and ActR-IIB (a gift of W. W. Vale) was done as described previously (40). Quantitation of the radioactivity associated with each band was done with a PhosphorImager 401E and ImageQuant software (Molecular Dynamics).

Nickel chromatography. Affinity-labeled cells were lysed in lysis buffer (20 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.5% Triton X-100) and protease inhibitors (36) and centrifuged to remove debris. Lysates were brought to 20 mM imidazole and incubated with Ni^{2+}-nitrilotriacetic acid (NTA)-agarose (Qiagen) for 1 h at 4°C, rinsed briefly three times, and then rinsed twice for 15 min each time with 20 mM imidazole in lysis buffer. Agarose-bound receptors were eluted with 200 mM imidazole.

Reverse transcription PCR (RT-PCR) analysis of endogenous ActR-II and IIB. RNA was prepared from R-1B cells by LiCl-urea precipitation as previously described (63). Total RNA (1 μg) was reverse transcribed into cDNA, using oligo(dT) priming (for ActR-II) or random priming (for ActR-IIB). cDNAs were amplified by using primers C and D for ActR-II or E and F for ActR-IIB. Amplification was performed on a Perkin-Elmer Thermal Cycler 9600, using a GeneAmp PCR kit (Perkin-Elmer). The cycling parameters were 94°C for 30 s, 37°C for 30 s, a 2-min ramp to 72°C, and then 72°C for the first three cycles, followed by 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min for 30 cycles. Amplified products were separated by agarose electrophoresis and transferred to a Genescreen Plus membrane (DuPont-New England Nuclear) in 0.4 M NaOH–0.6 M NaCl. DNA was fixed and hybridized overnight with ^{32}P-labeled (Megaprime DNA labeling kit; Amersham) PCR-derived fragments of ActR-II (nucleotides 1267 to 1494) or ActR-IIB (nucleotides 499 to 906) in 10% dextran sulfate–1% SDS–1 M NaCl–100 μg of sonicated

salmon sperm DNA per ml. Filters were washed three times in 2× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate [pH 7.0])–1% SDS at 65°C for 30 min and twice in 0.1× SSC at room temperature for 30 min.

Oligonucleotides used as PCR primers are as follows, with numbering starting on the start codon: primer C, ActR-II nucleotides 1267 to 1288, 5′-CCATCTCTTGAAGATATG CAGG-3′; primer D, ActR-II nucleotides 1474 to 1494, 5′-GACCACTGTTACAATGTCCTC-3′; primer E, ActR-IIB nucleotides 499 to 518, 5′-CCGGGATCCTACGGCCATG TGGACATCCA-3′; and primer F, ActR-IIB nucleotides 883 to 906, 5′-CCGCTCGAGATGCAGGTATGAGAGGCCTC GTGA-3′.

Cell proliferation assays. For dU incorporation assays, cells were seeded in 24-well plates at 50,000 cells per well. They were incubated overnight in regular growth medium and then placed in MEM plus 0.2% FBS and 50 μM $ZnCl_2$. After 5 h, cells were incubated for 18 h with appropriate concentrations of TGF-β1 or activin-A in MEM containing 0.2% FBS and 50 μM $ZnCl_2$. Cells were then incubated with 1 μCi of [^{125}I]dU (specific activity, 2,200 Ci/mmol; DuPont-New England Nuclear) per ml for an additional 4 h, washed three times with cold PBS, and fixed in 95% methanol for at least 1 h at 4°C. Fixed cells were washed twice with PBS and extracted with 1 N NaOH for at least 5 min. The extracts were collected and counted in a gamma counter. Each assay was carried out in triplicate.

For cell proliferation assays, cells were plated into 24-well plates, at 40,000 cells per well, in regular growth medium. After an overnight incubation, cells were shifted to MEM plus 0.2% FBS and 50 μM $ZnCl_2$ for 5 h. Cell were then incubated in MEM plus 5% FBS and 50 μM $ZnCl_2$ for 60 h, released from the dish, and counted with a Coulter Counter. During this final incubation, the cultures received 250 pM TGF-β1, 500 pM activin-A, or no addition as indicated.

Luciferase assay. One day after transfections, cells were seeded into 12-well plates, allowed to attach to the plastic for 4 h, incubated in MEM-f/g plus 0.2% FBS for 6 h, and then incubated in MEM-f/g plus 0.2% FBS for 14 h. During this final incubation, some cultures received 250 pM TGF-β1 or 500 pM activin-A as indicated. Cells were harvested, and luciferase activity in cell lysates was determined by using a luciferase assay system (Promega) as described by the manufacturer. Total light emission was measured during the initial 20 s of the reaction in a luminometer.

PAI-1 and fibronectin assays. Cultures that had reached 30 to 50% confluency in six-well plates were incubated overnight in MEM plus 10% FBS and 100 μM $ZnCl_2$. Cells were washed once with methionine-free MEM and incubated 4 h in 1 ml of methionine-free MEM containing 250 pM TGF-β1 or 500 pM activin-A as indicated in the figure legends. During the final 2 h of this incubation, 40 μCi of [^{35}S]methionine (1,180 Ci/mmol; Trans-^{35}S-Label; ICN Radiochemicals) was added to the medium. Monolayers were then chilled and washed at 4°C once with PBS and three times with a solution containing 10 mM Tris-HCl (pH 8), 0.5% sodium deoxycholate, and 1 mM phenylmethylsulfonyl fluoride, twice with 2 mM Tris-HCl (pH 8), and once with PBS. Extracellular matrix proteins were extracted from the plastic by scraping into electrophoresis sample buffer containing dithiothreitol. The samples were subjected to SDS-PAGE on a 10% polyacrylamide gel and analyzed by autoradiography. The plasminogen activator inhibitor 1 (PAI-1) band was identified by its characteristic mobility and induction by TGF-β (29).

To measure fibronectin production, cells grown for 24 h were induced by addition of 50 μM $ZnCl_2$ in medium containing 0.2% FBS, followed by the addition of 100 pM TGF-β or 500 pM activin-A for an overnight incubation. Cells were metabolically labeled with 50 μCi of [^{35}S]methionine per ml in methionine-free MEM for 2 h. The culture medium was collected, and fibronectin was isolated from it by binding to gelatin-Sepharose (Pharmacia) and analyzed as previously described (62).

Protein kinase assays and phosphoamino acid analysis. The TβR-I and TβR-II constructs of interest, all tagged with the HA epitope, were transiently transfected into COS-1 cells. On the second day after transfection, cells were lysed in TNE (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA) containing 0.5% Triton X-100 and immunoprecipitated by using an anti-HA antibody (12CA5; BAbCO) and protein A-Sepharose. The immunoprecipitates were washed four times with TNE containing 0.1% Triton X-100 and once with kinase assay buffer (50 mM Tris-HCl [pH 7.4], 10 mM $MgCl_2$, 1 mM $CaCl_2$). The beads were incubated at 37°C for 30 min in kinase buffer containing 60 μM ATP and 5 μl of [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham) in a total volume of 40 μl. The beads were washed three times with TNE containing 0.1% Triton X-100. Proteins were eluted by boiling in SDS-PAGE sample buffer and separated on an SDS–7% polyacrylamide gel. For phosphoamino acid analysis, the autophosphorylated TβR-I and TβR-II were eluted from the SDS-polyacrylamide gel, partially hydrolyzed, and analyzed by two-dimensional electrophoresis using the Hunter thin-layer electrophoresis system (HYL7000) as previously described (6).

RESULTS

Cloning of a human receptor related to the TβR-I receptor kinase. We cloned the human full-length version of a member of the TGF-β/activin/daf-1 receptor family whose ligand was previously unknown (Fig. 1). This receptor cDNA is a variant of *SKR2*, a cDNA cloned from the human hepatoma HepG2 cell line that encodes a cytoplasmic kinase domain atypical or incomplete at the C terminus (65) (Fig. 1A). To identify a version that would encode a typical kinase domain, we screened a human kidney cDNA library with a partial *SKR2* cDNA probe obtained by PCR with appropriate primers. This screen yielded a cDNA encoding a canonical kinase domain (Fig. 1A). On the basis of the functional properties described below, the receptor encoded by this human cDNA is termed ActR-IB. A partial cDNA encoding the cytoplasmic domain of ActR-IB (55) and a cDNA encoding the rat homolog of ActR-IB (20) have been recently reported by others as orphan receptors.

The predicted amino acid sequence of ActR-IB shows the structural features of a transmembrane serine/threonine kinase of the type I receptor subfamily (Fig. 1B). It contains a short extracellular domain with 10 cysteines arranged in a pattern that is similar to that of the human activin type I receptor ActR-I (2), its mouse homolog Tsk7L (11), the shared activin/TGF-β type I receptor TSR-I (2), the TGF-β type I receptor TβR-I (15), or the *Drosophila* activin type I receptor Atr-I (64). This pattern includes a C-terminal cluster, the cysteine box, that is also present in all type II receptors identified to date (10). Aside from the cysteine pattern, there is very little amino acid sequence similarity between the ectodomains of ActR-IB and TβR-I and even less between ActR-IB and the other activin type I receptor ectodomains (Fig. 1D).

The kinase domain of ActR-IB contains sequences characteristic of serine/threonine kinases (19). This domain is most closely related to that of TβR-I (15), showing 90% amino acid

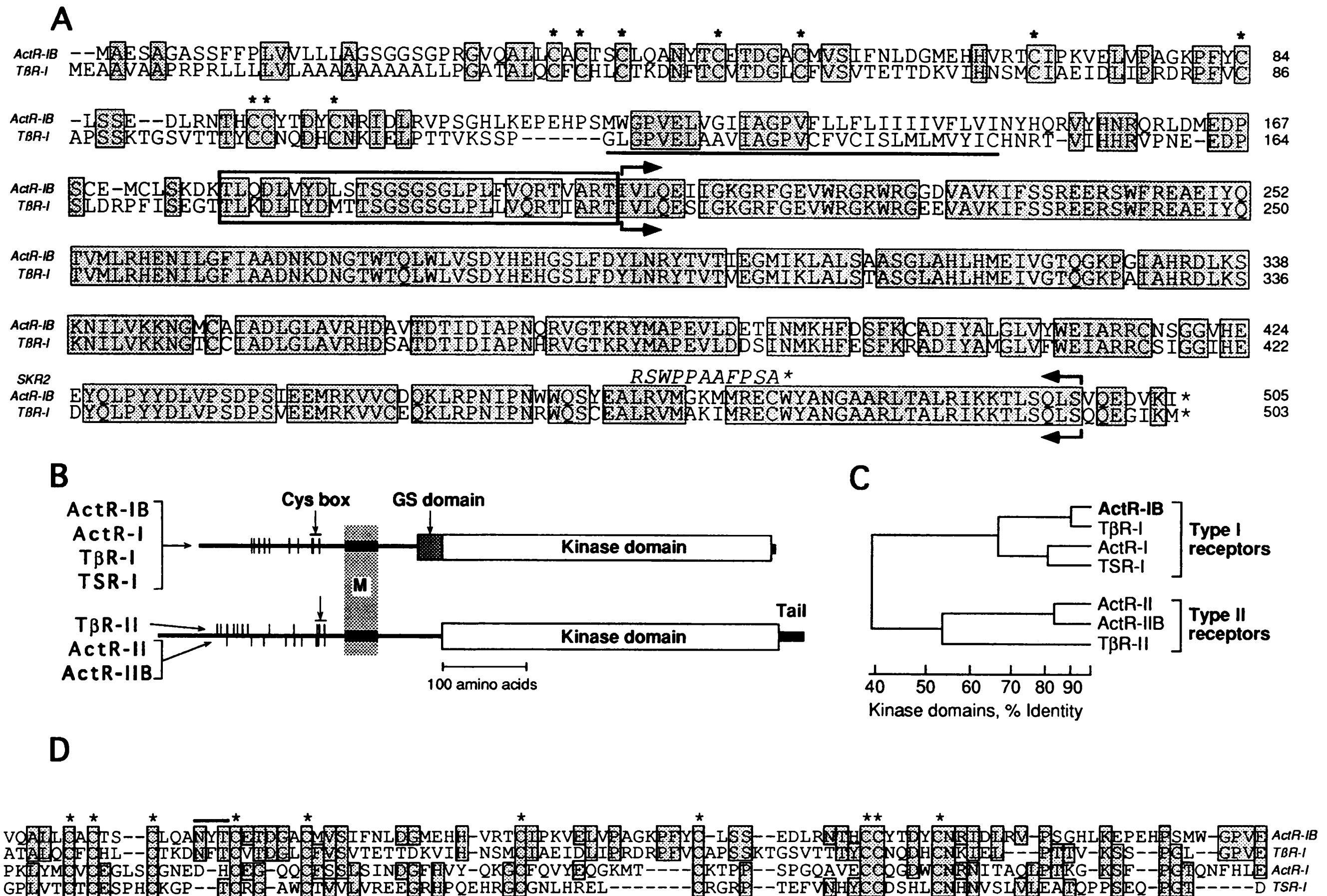

FIG. 1. ActR-IB sequence. (A) Alignment of the amino acid sequences of human ActR-IB and TβR-I (15). The extracellular cysteines (asterisks), putative transmembrane region (underlining), GS domain (open box), and kinase domain (arrow brackets) are indicated. The divergent sequence present in the human SKR2 variant of ActR-IB (65) is shown in italics. (B) Schematic representation of human type I and type II receptors for TGF-β and activin. Shown are the extracellular cysteines (vertical bars) and cysteine box (arrow), transmembrane region (M), the serine/threonine kinase domain, the GS domain of type I receptors, and the serine/threonine-rich C-terminal tail of type II receptors. The different patterns of extracellular cysteines in TβR-II versus ActR-II or IIB are indicated. (C) Sequence similarity between the kinase domains of mammalian activin and TGF-β type I and type II receptors. The relationship dendrogram was generated by using the Geneworks program. (D) Alignment of the type I receptor ectodomain regions showing amino acid identities. The sequences shown do not include the corresponding signal sequences and end with the last amino acid before the transmembrane region. Asterisks indicate conserved cysteines, and bars indicate potential N-linked glycosylation sites.

sequence identity (Fig. 1A and C) and 97% similarity. The level of identity to other members of the type I receptor subfamily ranges between 60 and 65%. Like other type I receptors, the kinase domain of ActR-IB is distantly related (35 to 40% amino acid sequence identity) to those of type II receptors for TGF-β (33), activin (3, 39, 41) (Fig. 1C), or bone morphogenetic protein (12). The kinase domain of ActR-IB is preceded by the GS domain, a 29-amino-acid region of unknown function that contains a characteristic SGSGSG sequence in the middle and is conserved in all known type I receptors from mammals and *D. melanogaster* (64).

Northern (RNA) blot analysis using a probe corresponding to the ectodomain indicated that ActR-IB is expressed in human brain, liver, lung and pancreas as an mRNA species of approximately 5 kb (data not shown).

ActR-IB has the ligand binding properties of an activin type I receptor. ActR-IB transfected into monkey COS-1 cells showed the ligand binding properties of an activin type I receptor (Fig. 2). When transfected alone, ActR-IB did not bind ^{125}I-activin-A or ^{125}I-TGF-β1, as determined by whole cell binding assays (not shown) or by receptor affinity-labeling assays (Fig. 2A). As previously shown (33, 39, 62), the TGF-β and activin type II receptors, TβR-II and ActR-II, bound their respective ligands in absence of type I receptors (Fig. 2A). Cotransfection of ActR-II supported activin binding to ActR-IB but not to TβR-I, whereas cotransfection of TβR-II supported TGF-β binding to TβR-I but little if any binding to ActR-IB (Fig. 2A). Cotransfection with the activin type II receptor isoform ActR-IIB (3) also supported activin binding to ActR-IB (data not shown).

Affinity labeling of ActR-IB/ActR-II cotransfectants with various concentrations of ^{125}I-activin-A under equilibrium binding conditions, followed by gel separation and quantitation of labeled receptor-ligand complexes, showed that binding reached saturation with 200 pM ^{125}I-activin-A for both receptors (Fig. 2B). These results are consistent with a K_D value of approximately 100 pM, which is similar to K_D values previously observed with ActR-II or IIB expressed alone or with ActR-I (2, 3, 39). Collectively, these results show that ActR-IB binds activin with high efficiency and specificity only in the presence

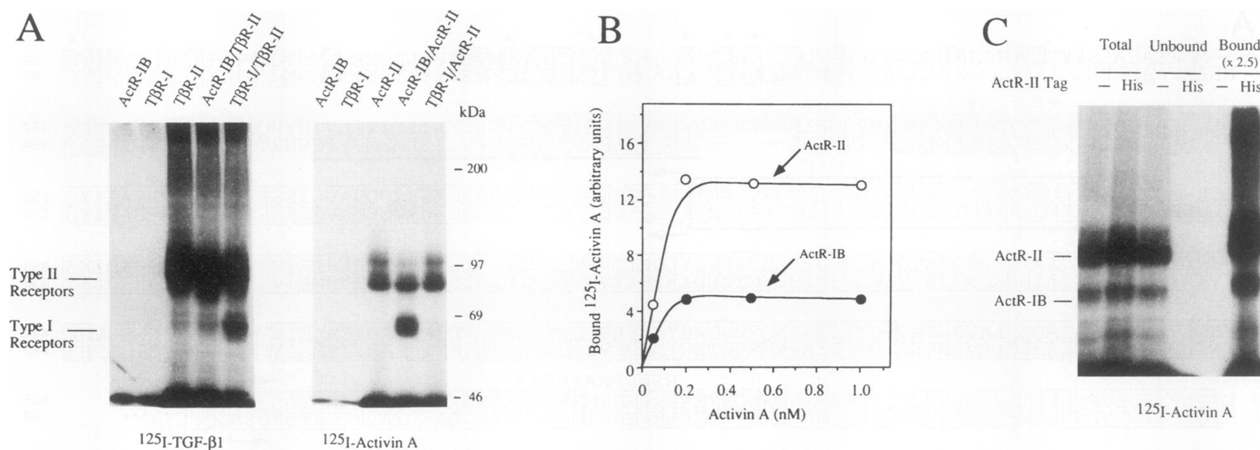

FIG. 2. Ligand binding properties of ActR-IB transfected into COS-1 cells. (A) ActR-IB binds activin-A only in the presence of ActR-II. COS-1 cells transfected with the indicated receptor cDNAs in the pCMV5 expression vector were affinity labeled by incubation with ^{125}I-TGF-β1 or ^{125}I-activin-A and cross-linking with disuccinimidyl suberate. Cell lysates were subjected to SDS-PAGE and autoradiography. (B) Activin-A binding to ActR-IB and ActR-II is saturable. COS-1 cells cotransfected with ActR-IB and ActR-II were incubated with the indicated concentrations of ^{125}I-activin-A. After cross-linking, SDS-PAGE, and autoradiography, the amount of signal associated with the radiolabeled products corresponding to ActR-II (open circles) and ActR-IB (closed circles) was quantitated with a PhosphorImager. (C) ActR-IB and ActR-II form a complex. COS-1 cells were cotransfected with ActR-IB and either wild-type ActR-II (−) or ActR-II tagged at the C terminus with a hexahistidine sequence (His). After affinity labeling with ^{125}I-activin-A, cell lysates were incubated with Ni^{2+}-NTA-agarose, and bound proteins were washed five times with lysis buffer containing 20 mM imidazole. Aliquots (1×) of the cell lysates (Total), supernatant after binding to Ni^{2+}-NTA-agarose (Unbound), and bound proteins from 2.5× aliquots of cell lysates (Bound) were subjected to SDS-PAGE and autoradiography.

of an activin type II receptor and that ActR-IB and TβR-I lack affinity for each other's ligand.

Another characteristic of type I receptors is that they form a noncovalent complex with the corresponding type II receptors (2, 15, 62, 64). To examine complex formation with ActR-IB, ActR-II was engineered with a hexahistidine sequence at its C terminus (ActR-II-His). This modification allows retrieval of the tagged protein from cell lysates by affinity chromatography on nickel-NTA-agarose. With this method, affinity-labeled ActR-IB was specifically and quantitatively recovered in association with ActR-II-His when the two were cotransfected into COS-1 cells (Fig. 2C). This result demonstrates a physical interaction between ActR-IB and ActR-II in the presence of ligand and argues that when ActR-IB is bound to activin, it remains in a trimolecular complex with ActR-II.

Interaction of TβR-I and ActR-IB with endogenous type II receptors. The kinase domains of ActR-IB and TβR-I are nearly identical, but the kinase domains of their corresponding type II receptors are very different from each other (42% amino acid sequence identity) (33). This observation prompted questions regarding the extent to which separate type I receptors with similar kinase domains may specify related responses when acting in concert with their respective ligands and type II receptors.

The Mv1Lu lung epithelial cell line and its mutants derivatives, selected for resistance to TGF-β action (5, 31), provide a system with which to test the functional properties of TGF-β receptors I and II (2, 4, 15, 61, 62). These cells have lost all known TGF-β responses, including growth inhibition and elevated fibronectin and PAI-1 production. The TGF-β-resistant mutants fall into three classes: R clones, which are defective in TβR-I; DR clones, which are primarily defective in TβR-II; and S clones, which display normal binding to both TGF-β receptors and must be defective in either the signaling function of the receptor or a postreceptor component essential for signal transduction. The R mutant clone R-1B has been characterized in detail (2, 5, 30, 62) and was used in most of these studies. When transfected into R-1B cells, TβR-I displayed TGF-β binding activity (Fig. 3A), presumably in concert with endogenous TβR-II.

In addition, R-1B cells can be used to test the functional properties of exogenous activin receptors because they express very low levels of endogenous receptors. Like parental Mv1Lu cells, R-1B cells showed very limited responsiveness to activin (2) (see below). They had no activin type I receptors, as detected by receptor affinity-labeling assays, although they showed a low level of ActR-IB mRNA by reverse transcription and PCR amplification of mRNA using ActR-IB-specific primers (data not shown). However, by affinity labeling (Fig. 3A), these cells showed a low level of activin type II receptors which were ActR-II and IIB, as determined by RT-PCR using with primers specific for ActR-II (39) or the alternatively spliced forms of ActR-IIB (3) (Fig. 3B).

These endogenous type II activin receptors supported a limited but significant level of ^{125}I-activin-A binding to transfected human ActR-IB (Fig. 3A) or human ActR-I (data not shown). Antibodies against ActR-II and ActR-IIB (40) coimmunoprecipitated ActR-IB from cells stably transfected with this receptor and affinity labeled with ^{125}I-activin (Fig. 3C), corroborating that ActR-II and ActR-IIB are expressed and can form a complex with ActR-IB in these cells. Transfection of TβR-I or ActR-IB into these cells diminished the labeling intensity of the corresponding endogenous type II receptors and altered somewhat the mobility of the cross-linked receptor products (Fig. 3A), a previously observed phenomenon of unknown significance (2, 11). As in COS-1 cells, ActR-I or ActR-IB transfected into R-1B cells did not bind ^{125}I-TGF-β1 (data not shown).

Participation of TβR-I and ActR-IB in growth-inhibitory signaling. Mv1Lu cells respond to TGF-β with an inhibition of DNA synthesis (Fig. 4A) as a result of cell cycle arrest in late G_1 phase (21, 28). This response is lost in R-1B cells (5), which, like parental Mv1Lu cells, show a very limited growth-inhibitory response to activin (Fig. 4A and B). We therefore used

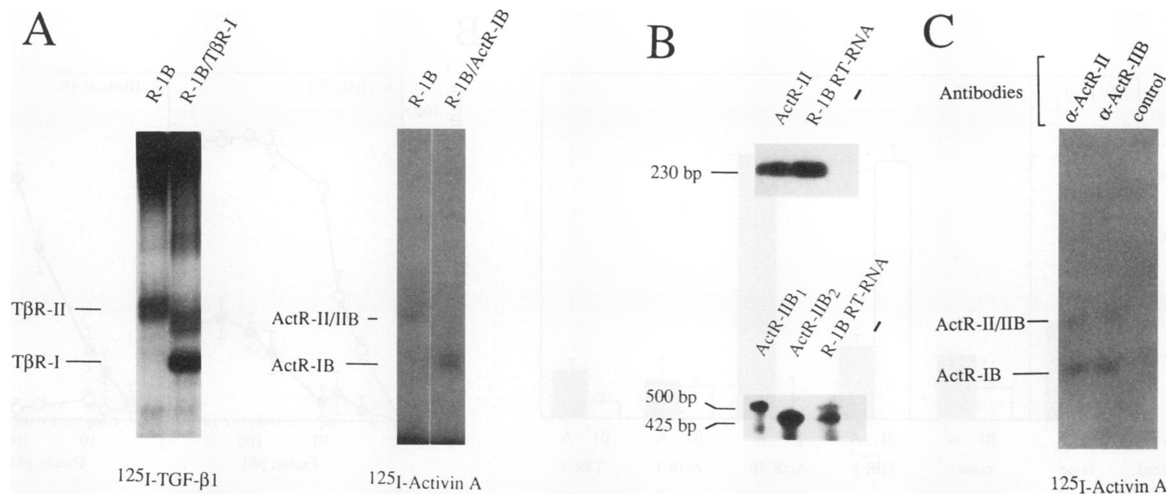

FIG. 3. Ligand binding to ActR-IB and TβR-I in R-1B cells expressing endogenous type II receptors. (A) ActR-IB binds activin-A in R-1B cells. R-1B cells and derivatives stably transfected with TβR-I or ActR-IB under the control of a metallothionein promoter were incubated overnight in medium containing 50 μM $ZnCl_2$. Cells were affinity labeled with ^{125}I-TGF-β1 or ^{125}I-activin-A as indicated. Labeled proteins were separated by SDS-PAGE and visualized by autoradiography. (B) Expression of activin type II receptors in R-1B cells. Equal amounts of RNA from R-1B cells were subjected to RT-PCR using primers specific for ActR-II or ActR-IIB (3). The RT-PCR samples, positive controls amplified with ActR-II cDNA (upper panel) or ActR-IIB$_1$ or ActR-IIB$_2$ cDNA (lower panel) as the template, and samples amplified without template (−) were hybridized to their respective ^{32}P-labeled cDNA probes. Sizes of the amplified products are indicated. (C) Complex formation between endogenous activin type II receptors and ActR-IB. R-1B cells stably transfected with ActR-IB were affinity labeled with ^{125}I-activin A, and cell lysates were immunoprecipitated with antibodies specific for ActR-II, ActR-IIB, or a control antigen.

R-1B cells to examine the ability of various type I receptors to confer growth-inhibitory responsiveness to TGF-β or activin.

R-1B cells were stably transfected with either TβR-I, TSR-I, ActR-I, or ActR-IB and tested for growth-inhibitory responses as asynchronously dividing cell populations. TGF-β1 caused an almost complete inhibition of DNA synthesis in cells transfected with TβR-I, as measured by incorporation of [^{125}I]dU into DNA (Fig. 4A). This effect was half maximal with 5 pM TGF-β1 (Fig. 4B). Cells transfected with TSR-I, ActR-I, or ActR-IB did not show this response to TGF-β1 (Fig. 4A). Likewise, activin-A caused an almost complete inhibition of DNA synthesis in cells transfected with ActR-IB (Fig. 4A and B). This effect was half maximal with approximately 50 pM activin-A (Fig. 4B). R-1B cells transfected with TβR-I, TSR-I, or ActR-I did not show any increase in growth-inhibitory responsiveness to activin compared with parental R-1B cells (Fig. 4A).

Cell number counts indicated that R-1B cell proliferation was not significantly affected by addition of TGF-β1 or activin-A to the cultures over a 60-h period (Fig. 4C). However, addition of TGF-β completely blocked the proliferation of R-1B cells transfected with TβR-I, and addition of activin-A had a similar effect on R-1B cells transfected with ActR-IB (Fig. 4C). Furthermore, R-1B cell clones cotransfected with ActR-IB and ActR-II showed, in general, a higher growth-inhibitory responsiveness to activin A than clones transfected with either receptor alone (Fig. 4D).

From this analysis, we concluded that TβR-I is a growth-inhibitory TGF-β receptor that acts in concert with TβR-II, and ActR-IB is a growth-inhibitory activin receptor that acts in concert with ActR-II or IIB. In the presence of the same type II receptors, the type I receptors TSR-I and ActR-I, whose kinase domains diverge from those of TβR-I and ActR-IB, were unable to signal growth inhibition.

TβR-I and ActR-IB specify a common set of transcriptional responses. To determine whether the similarities in signaling by TβR-I and ActR-IB extended beyond their growth-inhibitory responses, we determined their abilities to signal various transcriptional and extracellular matrix production responses that are typical of TGF-β in Mv1Lu cells. In the p3TP-lux reporter construct, luciferase expression is driven by a synthetic promoter that is highly inducible by TGF-β and to a lesser extent by activin (2, 62). Transient cotransfection of this construct provides an expedient way to survey the responsiveness of Mv1Lu cells and their mutant derivatives transfected with receptors of interest (4, 61, 62).

With p3TP-lux in parental Mv1Lu cells, luciferase activity was induced strongly by TGF-β1 but weakly by activin (Fig. 5A). R-1B cells cotransfected with p3TP-lux and pCMV5 vector did not respond to TGF-β. R-1B cells cotransfected with p3TP-lux and TβR-I showed a strong induction of luciferase activity in response to both TGF-β1 and TGF-β2 (Fig. 5A), indicating that TβR-I is involved in signaling this response and is a signaling receptor for the TGF-β2 isoform as well. In the absence of added ligands, R-1B cells cotransfected with ActR-IB and p3TP-lux showed elevated basal luciferase activity. The reason for this phenomenon is unknown. However, addition of activin caused a further elevation of reporter gene activity, reaching levels similar to those induced by TGF-β (Fig. 5A). Transfection of ActR-IB or TβR-I did not confer responsiveness to the other's ligand (Fig. 5A). Transfection of ActR-I into these cells has been shown to confer limited p3TP-lux reporter responsiveness to activin-A (2).

Similar assays done with DR-26 cells provided further evidence that the TGF-β-like responses induced by activin in ActR-IB transfectants did not involve endogenous TGF-β type II receptors. The endogenous TβR-II gene in DR-26 cells is inactivated by a point mutation that truncates this receptor in the transmembrane domain (62). DR-26 cells do not respond to TGF-β unless transfected with TβR-II (61, 62). When ActR-IB was transfected into this TβR-II-defective cell line, activin-A still induced a strong luciferase response (Fig. 5A).

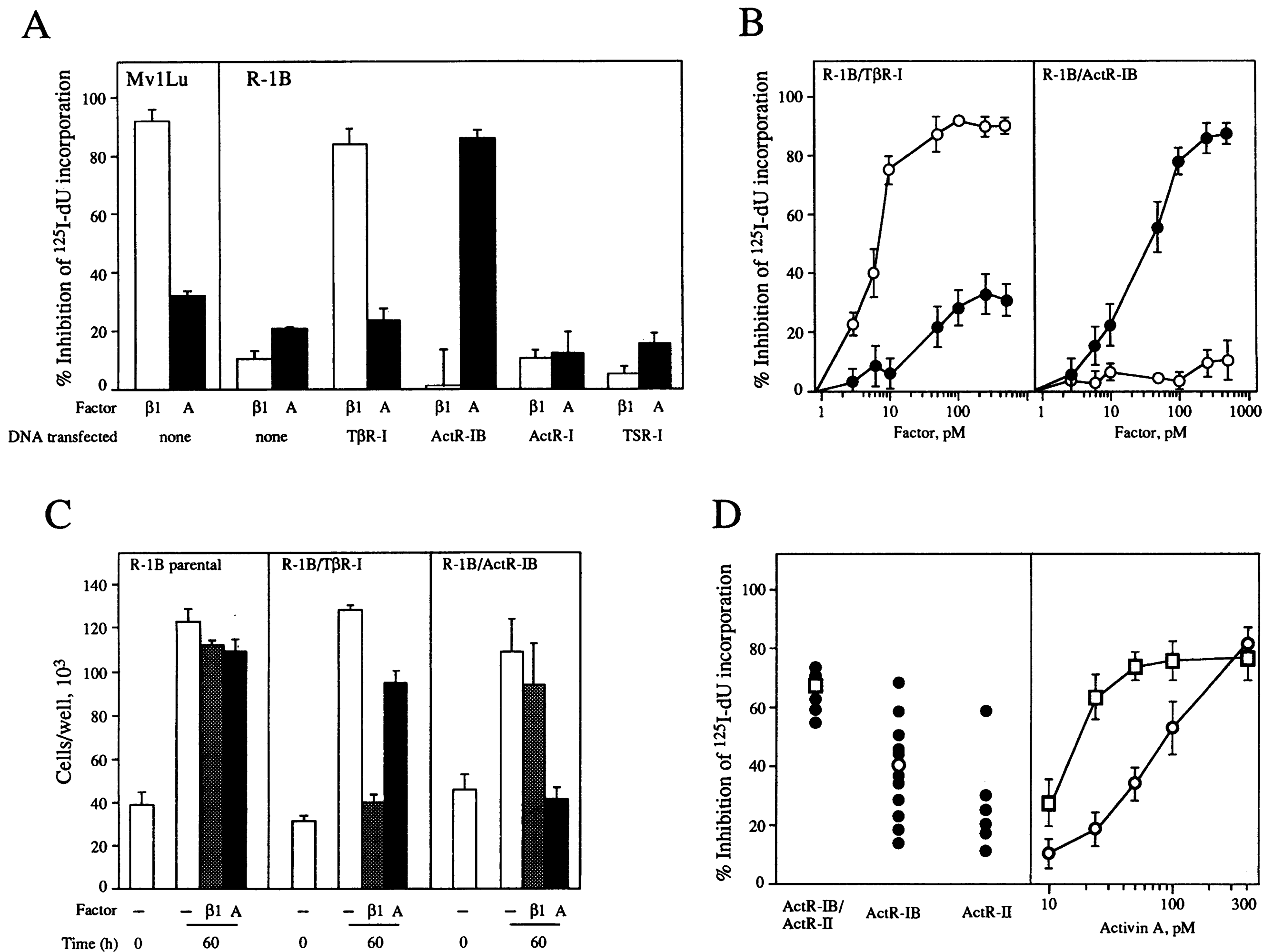

FIG. 4. TβR-I and ActR-IB mediate growth inhibition. (A) Inhibition of [^{125}I]dU incorporation by TGF-β1 or activin-A in parental Mv1Lu, parental R-1B, or R-1B stably transfected with the indicated type I receptor in a Zn^{2+}-inducible vector. Cultures were preincubated for 5 h in medium containing 50 μM $ZnCl_2$ and then incubated for 22 h in the same medium with 250 pM TGF-β1, 500 pM activin-A, or no additions. [^{125}I]dU was added during the final 4 h of incubation. The data are plotted as the average percent decrease in [^{125}I]dU incorporation ± standard deviation and are from one representative experiment with each point determined in triplicate. (B) TGF-β1 and activin-A dose-response curve for growth inhibition. R-1B cells stably transfected with TβR-I or ActR-IB were incubated with the indicated concentrations of activin-A (closed symbols) or TGF-β1 (open symbols) and processed as indicated above. (C) Inhibition of cell proliferation by TGF-β1 and activin-A. Parental R-1B or R-1B cells stably transfected with TβR-I or ActR-IB were seeded, left in growth medium overnight, and then incubated for 5 h in medium containing 50 μM $ZnCl_2$. Cells were then incubated for 60 h in medium containing 5% FBS and 100 μM $ZnCl_2$ alone (open bars) or with 250 pM TGF-β1 (shaded bars) or 500 pM activin-A (black bars). Cell counts were obtained in triplicate at the indicated times and are plotted as the average ± standard deviation. (D) Cotransfection of ActR-II with ActR-IB potentiates the growth-inhibitory responses to activin-A Clones of R-1B cells stably transfected with ActR-IB or ActR-II or cotransfected with ActR-IB and ActR-II were incubated with 50 pM of activin-A, and the inhibition of ^{125}I-dU incorporation was determined (left). Individual clones of ActR-IB/ActR-II (open squares) and ActR-IB (open circles) were incubated with the indicated concentration of activin-A (right) and processed as indicated above.

The physiological responses to TGF-β in Mv1Lu and other cells include an increase in fibronectin and PAI-1 production due to elevated transcription (22, 23, 25, 29). In Mv1Lu cells, activin is a very weak inducer of these responses (Fig. 5B and C). We analyzed the ability of the TGF-β and activin type I receptors to mediate fibronectin and PAI-1 responses in R-1B cells. Of the four receptors (TβR-I, TSR-I, ActR-I, and ActR-IB) tested in stable transfectants, only TβR-I restored responsiveness to TGF-β and only ActR-IB conferred responsiveness to activin (Fig. 5B and C and data not shown).

Type I receptor signals as a kinase. Previous evidence showed that TβR-II signals as a kinase. When produced recombinantly in bacteria and assayed in vitro, the TβR-II kinase domain shows autophosphorylating activity (33, 61). Mutations that disrupt the ATP binding site [TβR-II(K277R) construct] (62) or delete an insert from the kinase domain (61) yield TβR-II forms that are inactive as kinases and fail to signal.

When immunoprecipitated from transfected COS-1 cell lysates and subjected to a kinase assay, TβR-II and several coprecipitating products of unknown identity became phosphorylated (Fig. 6A). No phosphorylation was detected in similar experiments with the kinase-defective TβR-II(K277R) construct (Fig. 6A), suggesting that the activity associated with wild-type TβR-II was due to the receptor kinase. TβR-II autophosphorylation was essentially all on serine residues (Fig.

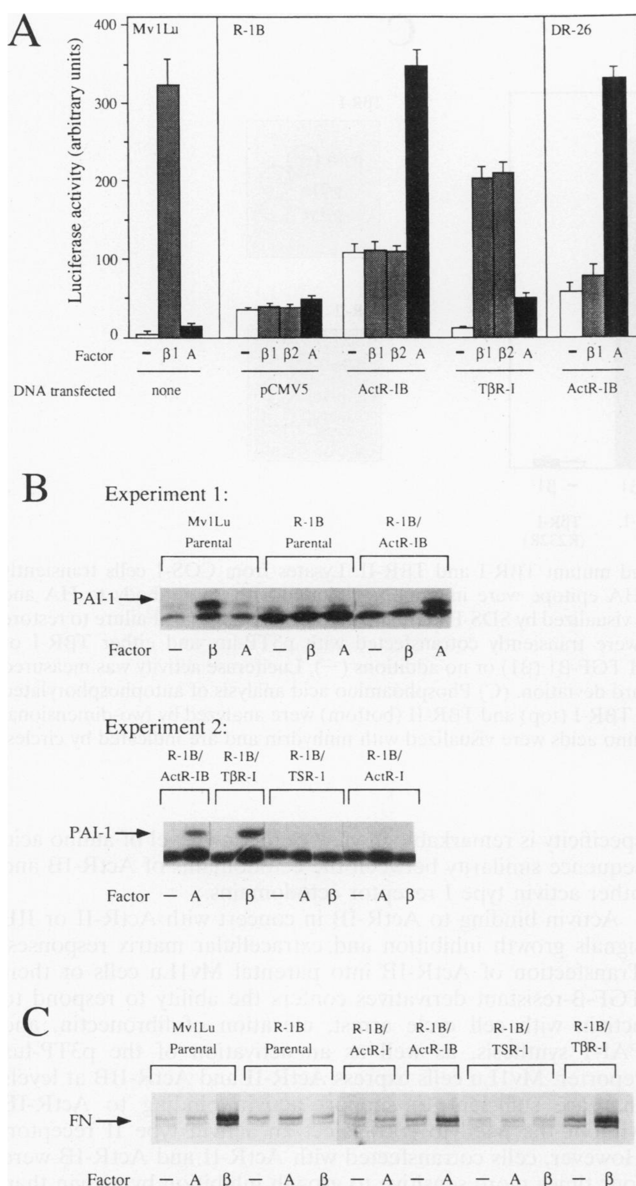

FIG. 5. TβR-I and ActR-IB mediate a common set of transcriptional responses. (A) Transcriptional activation of the p3TP-lux reporter by TGF-β and activin-A. Mv1Lu, R-1B, and DR-26 cells were transiently cotransfected with p3TP-lux reporter and the indicated type I receptor or vector alone. Cells were incubated for 14 h in medium containing 100 pM TGF-β1 (β1), 100 pM TGF-β2 (β2), 500 pM activin-A (A) or no additions (−). Luciferase activity was measured in cell lysates and is plotted as average of triplicate determinations ± standard deviation. (B) Induction of plasminogen activator inhibitor-1 (PAI-1) by TGF-β1 and activin-A. Sparse cultures of parental Mv1Lu, parental R-1B, or R-1B pools stably transfected with the indicated receptors were incubated overnight with medium containing 100 μM $ZnCl_2$. Cultures received 500 pM activin-A (A), 250 pM TGF-β1 (β), or no additions (−) and were labeled with [^{35}S]methionine. Secreted PAI-1 was visualized by SDS-PAGE and fluorography. The relevant regions of the fluorograms from two representative experiments are shown. (C) Elevation of fibronectin (FN) production by TGF-β1 and activin-A. The indicated parental or stably transfected cell lines were treated as for panel B except that incubations with factors were for 16 h. Cells were labeled with [^{35}S]methionine for the final 3 h of this incubation, and extracellular matrix proteins were detected by SDS-PAGE and fluorography. The relevant portion of the fluorogram is shown.

6C), which is in contrast with the ability of a recombinant cytoplasmic domain product to autophosphorylate on serine and threonine residues (33).

In this assay system, immunoprecipitated TβR-I showed serine/threonine kinase activity towards a protein of its own size (Fig. 6A and C). We introduced a point mutation that changed a lysine to arginine in the ATP binding site of the TβR-I kinase domain and is predicted to disrupt kinase activity (54). The resulting construct, TβR-I(K232R), bound TGF-β normally in concert with TβR-II when transfected in COS-1 cells (data not shown). However, this construct showed no kinase activity (Fig. 6A) and failed to restore responsiveness in R-1B cells, as determined with the p3TP-lux reporter assay (Fig. 6B). Thus, the kinase activities of both TβR-I and TβR-II are necessary for signal transduction by this receptor complex.

DISCUSSION

Recent searches for new members of the serine/threonine kinase receptor family have yielded various cDNAs whose products display binding properties of type I receptors for TGF-β and activin: they have to cooperate with TGF-β and activin type II receptors, respectively, in order to bind ligand. However, the functional properties of these molecules are largely unknown. We investigated the signaling activities of these products in cell lines that have low activin receptor levels and are defective in endogenous TGF-β receptors as a result of inactivating mutations. We found that only one of the two TGF-β type I receptors identified so far, TβR-I, mediates typical growth-inhibitory and extracellular matrix responses in concert with the TGF-β type II receptor. Having identified a new activin type I receptor, ActR-IB, whose kinase domain is nearly identical to that of TβR-I, we show that this but not other activin receptors mediates TGF-β-like growth-inhibitory and extracellular matrix responses in concert with activin type II receptors. These results provide insight into the role of each component in the heteromeric kinase receptors for TGF-β and activin.

TGF-β receptors that control cell cycle arrest and extracellular matrix expression. The human TβR-I/TβR-II complex is identified as a receptor that mediates two of the most characteristic responses to TGF-β, growth inhibition and stimulation of extracellular matrix protein expression. TβR-I was previously identified as a type I receptor on the basis of its ability to bind TGF-β in concert with TβR-II and restore stimulation of secretory protease inhibitor PAI-1 synthesis as well as activation of the p3TP-lux construct in Mv1Lu cell mutants defective in TβR-I (4, 15).

We show that TβR-I restores all TGF-β responses tested, including growth inhibition and various transcriptional responses. The presence of both TβR-I and TβR-II in Mv1Lu cells is required for the antimitogenic and transcriptional responses to TGF-β (15, 62; this report). Since kinase-defective TβR-I or TβR-II constructs do not rescue TGF-β responsiveness (62; this report), we conclude that signaling by this receptor complex requires the combined activity of both kinases.

Growth inhibition and extracellular matrix control by activin receptors. Growth inhibition and extracellular matrix control are not responses restricted to TGF-β. We have identified a human activin type I receptor that mediates these same responses in concert with activin type II receptors. This receptor is designated ActR-IB on the basis of its functional properties. ActR-IB represents the human full-length version of cDNAs cloned in searches for new members of the TGF-β/activin/daf-1 receptor family and whose ligand was unknown.

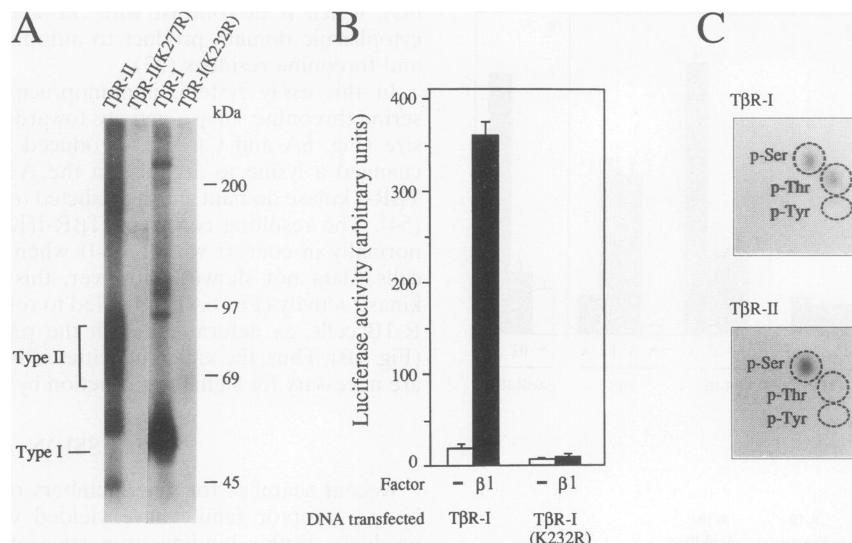

FIG. 6. TβR-I signals as a kinase. (A) Kinase activities of wild-type and mutant TβR-I and TβR-II. Lysates from COS-1 cells transiently transfected with the indicated constructs tagged with the influenza virus HA epitope were immunoprecipitated with an antibody to HA and incubated with buffer containing [γ-^{32}P]ATP. Phosphorylation products were visualized by SDS-PAGE and autoradiography. (B) Failure to restore TGF-β responsiveness by a kinase-defective TβR-I mutant. R-1B cells were transiently cotransfected with p3TP-lux and either TβR-I or TβR-I(K232R). Cells were incubated for 14 h in medium containing 250 pM TGF-β1 (β1) or no additions (−). Luciferase activity was measured in cell lysates and is plotted as average of triplicate determinations ± standard deviation. (C) Phosphoamino acid analysis of autophosphorylated TβR-I and TβR-II. The phosphoamino acids of in vitro-autophosphorylated TβR-I (top) and TβR-II (bottom) were analyzed by two-dimensional thin-layer chromatography electrophoresis. The positions of the phosphoamino acids were visualized with ninhydrin and are indicated by circles.

These include a partial ActR-IB cDNA designated ALK4 (55), the rat homolog of ActR-IB (20), and SKR2, which was cloned from the human hepatoma HepG2 cell line (65). The predicted SKR2 sequence is identical to that of ActR-IB except in the C-terminal portion of the kinase domain, where SKR2 encodes what appears to be a noncanonical or incomplete kinase.

ActR-IB has the structural properties of a type I receptor. It encodes a transmembrane protein with a short extracellular domain rich in cysteines and a cytoplasmic kinase domain. The extracellular cysteines are arranged in a pattern that is similar to that of other members of the type I receptor subfamily, irrespective of their ligand specificity. This pattern includes a C-terminal cluster, the cysteine box, that is also present in all type II receptors identified to date. The cytoplasmic kinase domain has traits characteristic of a serine/threonine kinase (19, 54). This domain is nearly identical (90% amino acid sequence identity, 97% similarity) to that of TβR-I and 60 to 65% identical to those of various human, rat, and *Drosophila* type I receptors for activin, TGF-β, and other, as yet unknown ligands. As with other type I receptors, the level of identity between the kinase domain of ActR-IB and the known type II receptors is very low, ranging between 35 and 40%. The kinase domain of ActR-IB is preceded by the GS region, a 29-amino-acid sequence that contains a characteristic SGSGSG motif in the middle and is conserved in all other type I receptors identified to date.

The binding properties of ActR-IB identify it as an activin type I receptor. Thus, when transfected alone in COS-1 cells, which contain very limited levels of endogenous type II receptors, ActR-IB does not bind activin-A or TGF-β1. When cotransfected with the mammalian activin type II receptor ActR-II or ActR-IIB, ActR-IB binds activin-A with high affinity but binds TGF-β1 inefficiently if at all when cotransfected with TβR-II. All activin-bound ActR-IB is found in a trimolecular complex with type II receptor. This binding specificity is remarkable in view of the low level of amino acid sequence similarity between the ectodomains of ActR-IB and other activin type I receptor ectodomains.

Activin binding to ActR-IB in concert with ActR-II or IIB signals growth inhibition and extracellular matrix responses. Transfection of ActR-IB into parental Mv1Lu cells or their TGF-β-resistant derivatives confers the ability to respond to activin with cell cycle arrest, elevation of fibronectin, and PAI-1 synthesis, as well as an activation of the p3TP-lux reporter. Mv1Lu cells express ActR-II and ActR-IIB at levels that are sufficient to support activin binding to ActR-IB without the need to cotransfect an activin type II receptor. However, cells cotransfected with ActR-II and ActR-IB were four times more sensitive to growth inhibition by activin than cells transfected with ActR-IB alone, indicating that this heteromeric complex mediates activin signaling. Furthermore, ActR-IB mediates responses to activin in cell mutants that are defective in either TGF-β type I (e.g., R-1B cells) or type II receptors (e.g., DR-26 cells), indicating that the TGF-β-like effects of activin do not involve TGF-β receptors.

Although previous studies on activin have focused mainly on its actions on pituitary, ovarian, and placental endocrine functions (34, 58, 59), erythroid differentiation (13, 66), and vertebrate embryo development (18, 50–52, 56, 60), limited growth-inhibitory responses to activin have also been described in Chinese hamster ovary cells (17), mouse 3T3 fibroblasts (27), human fetal adrenal cells (53), and vascular endothelial cells (43). TGF-β is thought to inhibit progression through G_1 phase by inhibiting the expression of c-Myc (45) and G_1 cyclins (14, 16), interfering with the activation process of a G_1 cyclin-dependent kinase complex via a 27-kDa inhibitor (26, 46), and, as a likely result, retaining Rb in its hypophosphorylated growth-suppressive state (28). The cell cycle block imposed by activin is likely to involve downstream events similar to those triggered by TGF-β.

TABLE 1. Human TGF-β and activin receptor repertoires

Subunit		Ligand	Responses
Type II	Type I		
TβR-II	TβR-I	TGF-β	Growth inhibition, fibronectin, PAI-1 expression
	TSR-I	TGF-β	Unknown
ActR-II or ActR-IIB	ActR-I	Activin	3TP transcription, other?
	ActR-IB	Activin	Growth inhibition, fibronectin, PAI-1 expression
	TSR-I	Activin	Unknown

The present results show that TGF-β and activin can signal a strong growth-inhibitory response when acting through the appropriate receptor isoforms in a susceptible cell type. However, we are not suggesting that the TβR-I/TβR-II complex and the ActR-IB/ActR-II (or ActR-IIB) complex are universal mediators of these responses in all cells. Additional studies are necessary to determine the extent to which the cellular context determines the responses that these receptors can elicit. We are also not suggesting that these complexes are mediators of all responses to TGF-β and activin. Clearly, other type I receptors for these factors exist, and at least one of them, ActR-I, can form a functional complex with ActR-II or ActR-IIB that stimulates transcription from the 3TP promoter construct (2). Since ActR-I did not stimulate PAI-1 expression, it is possible that its effect on the 3TP construct is mediated by the TRE elements rather than the PAI-1 promoter segment that are present in this construct.

Type I receptors determine the signaling specificity of heteromeric kinase receptor complexes. It is becoming clear that each type II receptor interacts with a repertoire of type I receptors generating complexes of different signaling capacity (Table 1). The known partners of TβR-II include TSR-I (2) and TβR-I (15). The known partners of ActR-II and ActR-IIB include ActR-IB, TSR-I, which is shared with TβR-II and signals as yet unknown responses, and ActR-I, which does not signal growth inhibition, fibronectin, or PAI-1 responses but signals activation of the p3TP-lux construct in cells transfected with ActR-II or ActR-IIB (2). The murine homolog, Tsk7L, can bind TGF-β (11); however, human ActR-I binds TGF-β inefficiently and fails to signal TGF-β responses (2; this report).

Since TGF-β and activin receptors contain two distantly related protein kinases that are both required for signaling, questions arise regarding the contribution of each receptor component to the signaling activity of the complex. TβR-I and ActR-IB mediate similar sets of responses even though their respective ligands and type II receptors are different. Mediation of this set of responses correlates with the presence of nearly identical kinase domains in TβR-I and ActR-IB. Other TGF-β or activin type I receptors, whose kinase domains are more divergent, do not mediate these responses even though they share type II receptors with TβR-I and ActR-IB. This leads to the conclusion that the signaling specificity of TGF-β and activin receptor complexes is determined primarily by the type I receptors.

Despite our emphasis on the role of the type I receptor, it is also clear that the kinase domain of type II receptors is essential in signaling. The evidence that the TβR-I/TβR-II complex, but not its individual components, mediates growth-inhibitory and transcriptional responses to TGF-β can be summarized as follows. In TβR-I-defective cells, TβR-II binds ligand but does not signal TGF-β responses (62). TGF-β responsiveness is restored by transfection of TβR-I into these cells (4, 15; this report). In TβR-II-defective cells, transfection of a TβR-II construct devoid of cytoplasmic domain allows TGF-β binding to TβR-I, but there is no response to TGF-β (61). TGF-β responsiveness is restored by transfection of wild-type TβR-II into these cells (24, 62). TβR-II harboring a point mutation in the ATP binding site as well as TβR-II containing truncations or deletions in the kinase domain support TGF-β binding to TβR-I but do not restore responsiveness to TβR-II-defective cell mutants (61, 62). Truncated TβR-II inhibits growth-inhibitory and transcriptional responses to TGF-β in a dominant negative fashion (7, 61), although others have reported dominant negative effects on the growth-inhibitory response only (9). Our present and previous results (61, 62) are consistent with the conclusion that the growth-inhibitory and extracellular matrix responses to TGF-β require the activity of both receptor components. Furthermore, our evidence does not support a model in which the transcriptional and growth-inhibitory responses would be separately mediated by receptor I and receptor II.

In addition to its role in signaling, the type II receptor subunit can bind ligand when expressed alone and therefore defines the ligand binding specificity of the complex. The ligand might induce or stabilize formation of the receptor complex, leading to activation of the kinases. However, the present results argue that the type I receptor kinase is key determinant of the signaling specificity of the complex. The different type I receptors that are available to the same type II receptor may each lead to a different set of responses, providing a mechanism to generate the complex multifunctionality of TGF-β and related factors.

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