Interaction of Transforming Growth Factor β Receptors with Apolipoprotein J/Clusterin†

Kumar B. Reddy,‡ Mary C. Karode,‡ Judith A. K. Harmony,§ and Philip H. Howe*,‡

Department of Cell Biology, Cleveland Clinic Research Institute, Cleveland, Ohio 44195-5245, and Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267-057

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ABSTRACT: Proteins mediating the transmission of the signal from an activated transforming growth factor β (TGFβ) receptor complex have not been identified. Using a yeast interaction screen to search for proteins that associate with the type II TGFβ receptor (RII), we isolated a protein which was identical to apolipoprotein J (apoJ)/clusterin. ApoJ interacts with both the type I (RI) and type II (RII) TGFβ receptors but does not interact with the epidermal growth factor (EGF) receptor. The interaction between RII and apoJ occurs through the C-terminal 127 amino acids of RII. Deletion of this region, which contains the kinase insert 2 domain, abrogates binding to apoJ. The binding of apoJ to either the RI and the RII receptors is direct, not requiring other proteins, and is not specific for the α or β subunit of apoJ since both subunits are effective in competing for binding. RI and RII fusion proteins are capable of precipitating the 60 kDa intracellular form of apoJ from [^{35}S]methionine-labeled cellular lysates, suggesting that this form of the protein may play some role in TGFβ signaling or TGFβ receptor processing.

The transforming growth factor β (TGFβ)[1] superfamily is a large group of multifunctional proteins that act on a wide variety of cell types to regulate cell proliferation, differentiation, and extracellular matrix formation (Massague, 1990; Roberts & Sporn, 1990). TGFβs elicit their effects through binding to three specific cell surface receptors, identified by cross-linking analysis and referred to as type I (RI), type II (RII), and type III or β-glycan (RIII) (Boyd & Massague, 1989). RIII is a membrane proteoglycan of 280−300 kDa with a short cytoplasmic domain that contains no apparent signaling motif but is very similar to the corresponding region of endoglin, a dimeric protein that also binds TGFβ, expressed in endothelial and mesangial cells (Lopez-Casillas et al., 1991; Wang et al., 1991). RIII and endoglin are thought to serve as extracellular reservoirs for TGFβ and in ligand presentation to the RI and RII signaling receptors (Lopez-Casillas et al., 1993). RI and RII are glycoproteins of 53 and 75 kDa, respectively (Boyd & Massague, 1989). Sequence and functional complementation analyses have shown that both are transmembrane serine/threonine kinase receptors required for TGFβ signaling (Lin et al., 1992; Wrana et al., 1992; Franzen et al., 1993). RII belongs to an expanding family of related serine/threonine kinase receptors which includes the activin type II receptors from vertebrates and the bone morphogenetic protein type II receptor daf-4 in *Caenorhabditis elegans* (Mathews & Vale, 1991; Estevez et al., 1993). RI also belongs to this same family of serine/threonine kinase receptors, and to date six closely related RI receptors have been cloned (ten Dijke et al., 1994).

The molecular mechanism of activation of the serine/threonine kinases for TGFβ remains unclear. The formation of both heteromeric and homomeric complexes between the two receptor types has been proposed (Wrana et al., 1992; Chen & Derynk, 1994; Henis et al., 1994). Earlier studies demonstrated that a heteromeric complex between RII and RI is required for signaling. It was shown that RI binds ligand only in the presence of RII; RII can bind ligand in the absence of RI, but it requires RI to signal (Wrana et al., 1992). Interaction between the two receptor types was further demonstrated by coimmunoprecipitation experiments. Antibodies specific for each receptor type precipitated both ^{125}I-TGFβ cross-linked RI and RII receptors (Wrana et al., 1992; Franzen et al., 1993; Moustakas et al., 1993; Inagaki et al., 1993; ten Dijke et al., 1994). Receptor phosphorylation has been proposed as an activation mechanism in this heteromeric receptor model. RII autophosphorylation on serine and threonine residues has been demonstrated *in vitro* by using an RII fusion protein (Lin et al., 1992), and its kinase activity has been shown to be required for signaling (Wrana et al., 1992). Deletion of the insert 2 region within the kinase domain of RII (residues 490−508) results in a receptor which binds ligand and supports ligand binding to and complex formation with the RI receptor, but does not mediate TGFβ signaling (Weiser et al., 1993). More recently, it has been proposed that RII is a constitutively active kinase and that ligand binding induces complex formation and trans-phosphorylation of RI by RII, resulting in an activated signaling complex (Wrana et al., 1994). Homomeric models for signaling have also been proposed. RII and RIII have been shown to form homooligomers in the absence or presence of ligand. These preexisting homomeric complexes can bind TGFβ, and in addition to

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* Address correspondence to this author at the Department of Cell Biology, Cleveland Clinic Research Institute, Cleveland, OH 44195. Telephone: 216/445-9750. FAX: 216/444-9404.

‡ Cleveland Clinic Research Institute.

§ University of Cincinnati.

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[1] Abbreviations: apoJ, apolipoprotein J; TGFβ, transforming growth factor β; RI, RII, and RIII, transforming growth factor β receptor types I, II, and III, respectively; Tsk-7L and ALK-5, transforming growth factor β receptor type I cDNAs.

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the extracellular and transmembrane domains, the cytoplasmic regions of the receptors can interact with each other (Henis et al., 1994).

The intracellular signaling mediators which are recruited to an activated TGFβ receptor complex have not been identified. It has recently been reported, using the yeast two-hybrid system, that the intracellular domain of the RI receptor specifically interacts with the immunophilin FKBP-12, an intracellular binding protein for the immunosuppressive macrolides FK506 and rapamycin (Wang et al., 1994). All the RI receptor subtypes interacted with FKBP-12, but the intracellular domain of the RII class of receptors failed to interact in this genetic screen. While the physiological significance of such an interaction remains to be clarified, it suggests that the TGFβ and rapamycin signaling pathways share common intracellular mediators. In the present study, we have used the yeast two-hybrid approach to identify proteins which interact with the cytoplasmic domain of the RII TGFβ receptor. Screening of both a human placental and human brain cDNA library resulted in the isolation of a common cDNA which was identical to the DNA sequence encoding human apolipoprotein J (apoJ) or clusterin. Complemented with *in vitro* binding and immunoprecipitation studies, our data show that apoJ interacts with the cytoplasmic domain of both the RI and RII TGFβ receptors.

EXPERIMENTAL PROCEDURES

Library Screening. Human placenta and brain Matchmaker cDNA libraries and other vectors were purchased from Clontech. cDNA for human TGFβ-RII was a gift from Dr. H. F. Lodish. TGFβ-RI receptor cDNAs, Tsk-7L and ALK-5, were obtained from Drs. R. Derynk and K. Miyazono, respectively. The two-hybrid assay was used to screen for cDNAs encoding proteins able to interact with RII. Cytoplasmic domains of RII (residues 195−567), Tsk-7L (residues 149−509), and ALK-5 (residues 151−503) were PCR-amplified (the conditions for the PCR amplification were as follows: 95 °C for 15 min followed by 30 cycles of 95 °C for 15 s; 55 °C for 30 s; and 72 °C for 90 s and 65 °C for 10 min) and cloned into the pGBT9 GAL4 DNA binding domain vector. The cytoplasmic domain of RII was amplified using the forward primer 5′GAATTCCAGAAGCTGAGTTCA3′ and the reverse primer 5′GGATCCCTATTTGGTAGTGTT3′. Tsk-7L was amplified using primers 5′GAATTCTTT AAGAGAC GCAAT3′ (forward primer) and 5′GGATCCACAGTCAGTTTTTAA3′ (reverse primer). ALK-5 was amplified using primers 5′CCCGGGGCGCACTGTCATTCACCATCGA3′ (forward primer) and 5′CTGCAGTTACATTTTGATGCCTTCCTG3′ (reverse primer). Yeast strain HF7c was first transformed with pGBT9 DNA binding domain vector containing RII cytoplasmic domain, and colonies were selected on SC-Trp plates. A single Trp^+ colony was amplified and transformed with a human placental cDNA library in the pGAD10 expression vector, and the transformants were selected at 30 °C on SC-Trp, Leu, and His containing 25 mM 3AT. After 7−10 days, His^+ colonies were screened for β-galactosidase activity by using a filter assay (Fields & Song, 1989). cDNAs from the positive colonies were isolated and used for secondary screening against RII. The positive cDNAs from the secondary screening were retested for their "bait" specificity with unrelated cDNAs in a tertiary screening. These positive cDNAs were sequenced and compared with the known sequences from Genbank.

Expression and Purification of Gst-Fusion Proteins. The cytoplasmic domain of RII corresponding to amino acid residues 72−567 (amplified using the forward primer 5′GGATCCTGCAGCATCACC3′ and the reverse primer 5′GAATTCTTTGGTAG TGTT3′), and 271−567 (amplified using the forward primer 5′GGATCCGAGACAGTGGCA3′ and the reverse primer 5′GAATTCTTTGG TAGTGTT3′) and various deletion mutants corresponding to residues 271−440 (amplified using the forward primer 5′GGATCCGAGACAGTGGCA3′ and the reverse primer 5′GAATTCCTCAGCATTCTCCAA3′) and 439−567 (amplified using the forward primer 5′GGATCCGCTGAGTCCTTC3′ and the reverse primer 5′GAATTCTTTGGTAGTGTT3′) as well as the intracellular domain of Tsk-7L (RI), corresponding to residues 149−509, were PCR-amplified and cloned into pGEX-2T vector (Pharmacia). For purification of the various Gst-proteins, cultures growing in log phase were induced with 500 mM IPTG for 2 h. Cell pellets were resuspended in lysis buffer (PBS containing 150 mM NaCl and 1% Triton X-100), sonicated for 2 × 15 s on ice, and clarified by centrifugation. Glutathione−sepharose beads were added to the supernatants, and adsorption of the fusion proteins to the beads was allowed to occur for 15 min at room temperature. Beads were washed 3 times in lysis buffer and frozen in aliquots (Smith & Johnson, 1988).

In Vitro Binding Studies. For *in vitro* binding assays, human plasma was diluted in PBS. Various Gst-fusion proteins adsorbed to glutathione beads were incubated with 1:3-diluted human plasma for 4−6 h at 4 °C. The bound proteins were washed 4 times in wash buffer (PBS containing 0.2% Tween-20), and analyzed by SDS−PAGE and immunoblotted with anti-apoJ antibodies and ^{125}I-protein A. ApoJ was purified from human plasma to homogeneity by the methods described previously (de Silva et al., 1990a), and 10 μg was radioiodinated by using the iodine monochloride method (MacFarlane, 1958). ^{125}I-ApoJ (1.4×10^5 cpm; specific activity 2×10^7 cpm/μg) was incubated with various beads in the presence of either 0−20 μg of unlabeled apoJ or 0−50 μg of its individual subunits at 4 °C for 4−6 h. The beads were washed 4 times with wash buffer; results were analyzed by SDS−PAGE followed by autoradiography. The purification of α and β subunits has been described previously (de Silva et al., 1990c).

Metabolic Labeling and Immunoprecipitations. HepG2 cells growing in 100 mm tissue culture plates were starved in methionine-deficient medium for 1 h. Cells were pulse-labeled for 25 min with 200 μCi/mL of [^{35}S]methionine in methionine-deficient medium. Cells were lysed in 50 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 0.2% NP-40. The clarified cell lysates were subjected to immunoprecipitation, using anti-apoJ antisera (Burkey et al., 1991), or precipitated with Gst-fusion constructs coupled to agarose beads.

RESULTS AND DISCUSSION

Recently, it has been demonstrated that the RI receptor, in a genetic screening analysis using the two-hybrid system, interacts with the rapamycin binding protein FKBP-12 (Wang et al., 1994). The physiological significance of such binding is unclear; however, the fact that both TGFβ (Howe et al., 1991) and rapamycin (Morice et al., 1993) inhibit cell cycle progression late in G1 and both have negative effects on the cyclin/cdk pathway has led to the notion that TGFβ and

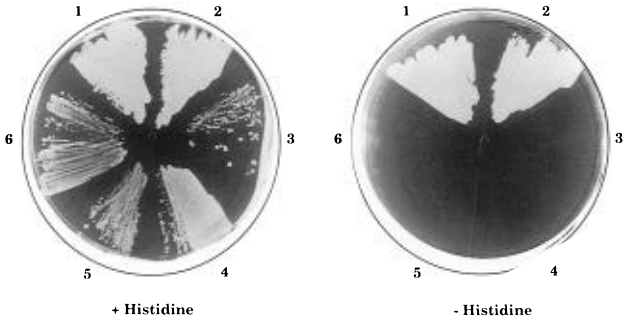

FIGURE 1: Strategy for isolating cDNAs encoding RII-associated proteins. HF7c yeast cells were cotranformed with apoJ in Gal4 activation domain vector pGAD10 along with one of the following DNAs in Gal4 DNA binding vector pGBT9. The transformants were selected on synthetic complete medium ±histidine. Growth on SC minus His plates indicates interaction between two proteins: (1) apoJ+RII; (2) apoJ+RI; (3) apoJ+cdk2; (4) apoJ+SNF1; (5) apoJ+lamin C; and (6) apoJ+pGBT9 vector.

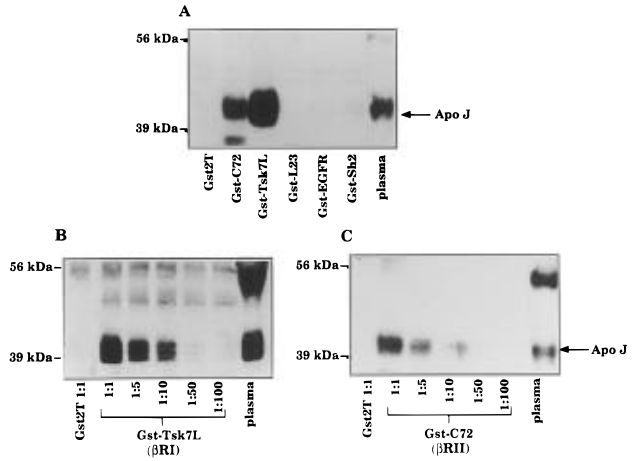

FIGURE 2: Characterization of the interaction between TGFβ-receptors and apoJ. Panel A: Various Gst-proteins were incubated with 1:3-diluted plasma for 4−6 h at 4 °C. The beads were washed 4 times with wash buffer, and bound proteins were separated by SDS−PAGE under reducing conditions. Immunoblot analysis was performed using anti-apoJ antibodies and ^{125}I-protein A. Similarly, 1:1−1:100-diluted plasma was incubated either with Gst-Tsk7L(RI) (panel B) or with Gst-C72(RII) (panel C), and bound apoJ was identified by immunoblotting. In all cases, 5 μL of plasma was loaded on the gels as positive control.

rapamycin share common signaling components. We also utilized the two-hybrid system (Fields & Song, 1989) to identify receptor-associated proteins but, instead of the RI TGFβ receptor, performed our genetic screen with the cytoplasmic domain of the RII receptor. The entire cytoplasmic domain of RII (residues Q195−K567) was fused with the GAL4−DNA binding domain and used as "bait". Screening of $\sim 2 \times 10^6$ clones from a human placental cDNA library yielded 10 positive clones. Two of the positives clones had ~1.6 kb inserts. These two cDNAs were sequenced and found to be identical. Their DNA sequences were compared with known sequences in GenBank, and identified as human testosterone-repressed prostate message 2 (TRPM-2)/apolipoprotein J (apoJ) (de Silva et al., 1990b; Wong et al., 1993). Homologues of apoJ/TRPM-2 identified so far include clusterin in sheep (Blaschuk et al., 1983), sulfated glycoprotein 2 (SGP-2) in rat (Collard & Griswold, 1987), and glycoprotein 80 (gp80) in dog (Hartmann et al., 1991). Other positive clones had very short reading frames with no significant sequence homology among them. We also independently screened a human brain cDNA library with the "RII bait" and were successful in isolating apoJ cDNA, demonstrating that this is not a library-dependent phenomenon. The specificity of the interaction between apoJ and RII was further investigated (Figure 1). TGFβ receptor RII and also RI (Tsk7L subtype) (Ebner et al., 1993) were specific in their interaction with apoJ. Other unrelated cDNAs, encoding cdk2, SNF1, or lamin C, commonly used as controls in the two-hybrid system, failed to interact with apoJ. Another family member, the Alk5 subtype (Franzen et al., 1993), was also positive in its ability to interact with apoJ (data not shown).

We wished to confirm the interaction between the RII TGFβ receptor and apoJ in an assay independent of the two-hybrid system. Since apoJ is present in high concentration in human plasma (50−125 μg/mL), we incubated plasma with various Gst-fusion proteins and determined their ability to interact with apoJ. Human plasma was diluted 1:3 with PBS and incubated with Gst-fusion proteins coupled to glutathione−agarose beads for 4−6 h at 4 °C. The bound proteins were washed, separated by SDS−PAGE under reducing conditions, and immunoprobed with anti-apoJ antibodies and ^{125}I-protein A. As shown in Figure 2A, only the TGFβ receptor Gst-fusions (Gst-C72, RII; Gst-Tsk7L, RI) interacted with apoJ. This form of apoJ represents the secreted and cleaved form of the protein (de Silva et al., 1990b). No apoJ was absorbed from plasma by the intracellular domain of a tyrosine receptor kinase, the Gst-EGFR (Koland et al., 1990), nor does binding occur with a Gst-SH2 domain of murine hematopoietic cell phosphatase (HCP) (Yi et al., 1992), or Gst-ribosomal fusion protein L23 (Suzuki & Wool, 1993). This interaction is specific for the TGFβ receptor family of serine/threonine kinases; positive interactions were detected with two RI receptors, Tsk-7L and Alk5 (data not shown), and the RII receptor. Also, apoE, an apolipoprotein with some characteristics shared with apoJ, failed to interact with TGFβ receptor Gst-fusion proteins under the same experimental conditions (data not shown). It is further shown in Figure 2B,C that the RI receptor binds with greater affinity to apoJ than does the RII receptor, but the binding of both receptor types exhibits saturable binding kinetics.

ApoJ may directly interact with TGFβ receptors or require additional proteins. In order to determine whether a bridging protein is involved in TGFβ receptor association with apoJ, ^{125}I-apoJ was incubated with the Gst-RI fusion protein in the absence or presence of competing, unlabeled apoJ for 4−6 h at 4 °C. The beads were washed and the results analyzed by SDS−PAGE followed by autoradiography. It is clear from Figure 3A that the RI and RII receptors specifically associated with ^{125}I-apoJ and that its interaction was competed by unlabeled apoJ in a dose-dependent fashion. The bands corresponding to each lane were quantitated, and relative affinities of RI and RII for apoJ were determined. RI exhibited higher affinity for apoJ compared to RII (166 and 70 pM cold apoJ inhibited 40% of ^{125}I-apoJ binding to RI and RII, respectively). These results are in agreement with those shown in Figure 2B,C. In subsequent experiments (Figure 3B), we determined that both the α and β subunits of apoJ competed with the radiolabeled protein for binding to Tsk-7L. However, it required approximately 2-fold more of the individual subunits to compete as effectively as the dimeric apoJ. Similar results were obtained using RII (data not shown). Although the sequences in apoJ subunits involved in the interaction with TGFβ receptors have to be determined, there exists a certain

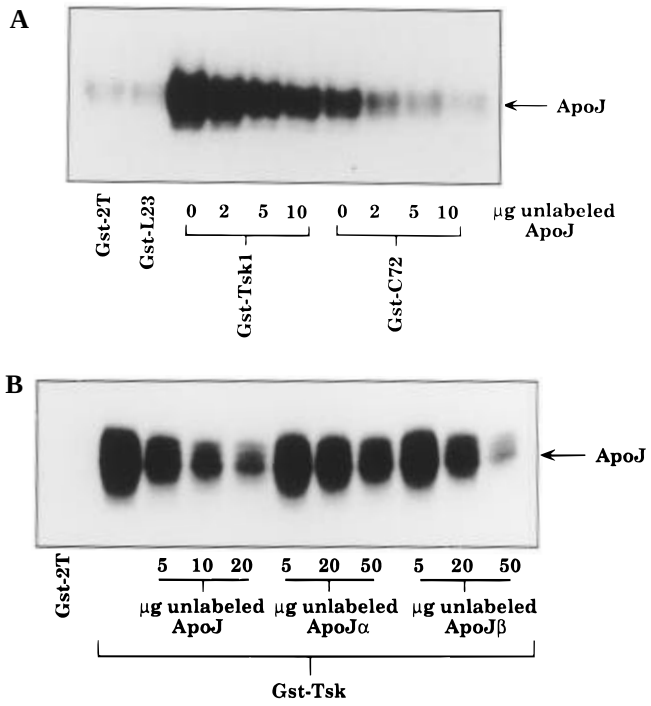

FIGURE 3: ApoJ directly interacts with TGFβ-receptors. Panel A: 1.4×10^5 cpm of ^{125}I-apoJ (specific activity 2×10^7 cpm/μg) was incubated with Gst-Tsk7L (R1) or Gst-C72(RII) agarose beads in the presence of competing, unlabeled apoJ. Incubations were carried out for 4−6 h at 4 °C. Precipitated beads were washed as described in under Experimental Procedures and analyzed by SDS−PAGE and autoradiography. Panel B: 1.4×10^5 cpm of ^{125}I-apoJ (specific activity 2×10^7 cpm/μg) was incubated with Gst-Tsk7L (R1) agarose beads in the presence of competing, unlabeled apoJ α or β subunits and processed as described in panel A.

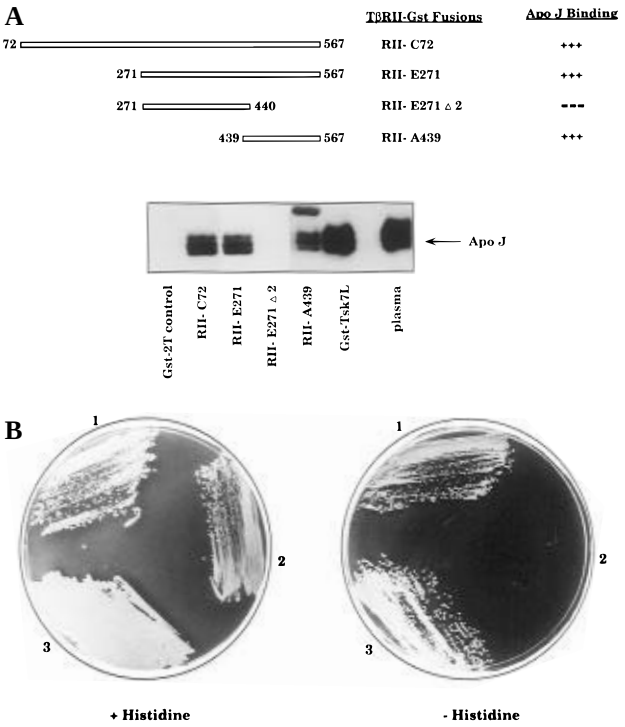

FIGURE 4: C-terminal portion of TGFβ RII is sufficient to recognize apoJ. Panel A: 1:3-diluted plasma was incubated with various Gst-deletion mutants of RII, and immunoblotting was performed as described in Figure 2. RII-C72 represents the entire intracellular domain (positions 72−567); RII-E271 lacks the N-terminal region of the intracellular domain (positions 271−567); RII-E271Δ2 lacks the C-terminal 127 amino acids (positions 440−567); and RII-A439 represents the C-terminal 127 amino acids (positions 440−567). Panel B: HF7c yeast cells were cotransformed with apoJ in Gal4 activation domain vector along with one of the RII mutants in pGBT9 vector. Transformants were selected on synthetic complete medium ±histidine: (1) apoJ+RII-195−567(entire cytoplasmic domain); (2) apoJ+RII-195−440 (lacks insert 2 and C-terminus); and (3) apoJ+RII-439−567 (insert 2 and C-terminus).

degree of structural as well as functional similarities between both subunits. Both subunits have also been shown to inhibit complement-mediated erythrocyte lysis *in vitro*, albeit less effectively than dimeric apoJ (Tschopp et al., 1993), with the activity shown to be localized to amphipathic helical domains 150−178 (α subunit) and 221−248 (β subunit) (personal communication; Kelso et al., 1995). These results demonstrate that the interaction between TGFβ receptors and apoJ is specific, dose-dependent, and direct, not requiring a bridging protein.

TGFβ RII receptor is a serine/threonine kinase which contains two inserts in its kinase domain and a C-terminal tail which is rich in serine and threonine (Weiser et al., 1993). It has previously been shown that alterations or deletions in the kinase insert 1 region or the C-terminal tail have little effect on ligand-induced responses. However, deletions in the kinase insert 2 domain yield a receptor which is unable to mediate TGFβ-induced responses. To determine the region of the TGFβ receptor necessary for binding, a series of deletion mutants of the RII receptor were created and expressed as Gst-fusion proteins. These were then analyzed for their ability to bind and precipitate apoJ as described above. As demonstrated in Figure 4A, the Gst-RII encoding the entire intracellular domain (and its transmembrane domain as well), RII-C72, bound apoJ. Deletion of the transmembrane domain and the N-terminal region of the intracellular domain (positions 72−271; RII-E271) had no effect on apoJ binding (lane 3). Deleting the C-terminal 127 amino acids (positions 440−567) from RII-E271, RII-E271Δ2, removing the insert 2 and terminal tail domains, abolished apoJ binding (lane 4). A short 127 amino acid peptide (positions 439−567), representing the insert 2 domain and the C-terminal tail, RII-A439, was sufficient to restore apoJ binding (lane 5). Thus, it appears that insert 2 region and/or the C-terminal region of the kinase participates in apoJ binding.

The *in vitro* results with apoJ and Gst-deletion mutants were confirmed by studies performed in the two-hybrid system and presented in Figure 4B. PCR-amplified products, representing the entire RII intracellular domain (positions 195−567; Q195), a deletion mutant lacking insert 2 and the C-terminal tail (positions 195−440; Q195Δ2), and a deletion mutant containing only the insert 2 and C-terminal domain (positions 439−567; A439) were ligated into the pGBT9 Gal4 binding domain vector and transformed into yeast cells containing apoJ in the pGAD10 Gal4 activating domain vector. Positive interactions were detected with either the entire intracellular domain, mutant Q195, or the insert 2 and C-terminal portion of RII, mutant A439 (see 1 and 3 in Figure 4B). The mutant lacking the insert 2 and C-terminal domain of RII, Q195Δ2, failed to interact with apoJ (see 2 in Figure 4B). These results demonstrate by two independent assays that the C-terminal 127 amino acids of the RII receptor, containing the insert 2 domain and the C-terminal serine/threonine-rich domain, are sufficient for interaction with apoJ.

In parallel with these *in vitro* studies, we wished to determine whether these interactions between apoJ and TGFβ receptors can occur in mammalian cells. We performed

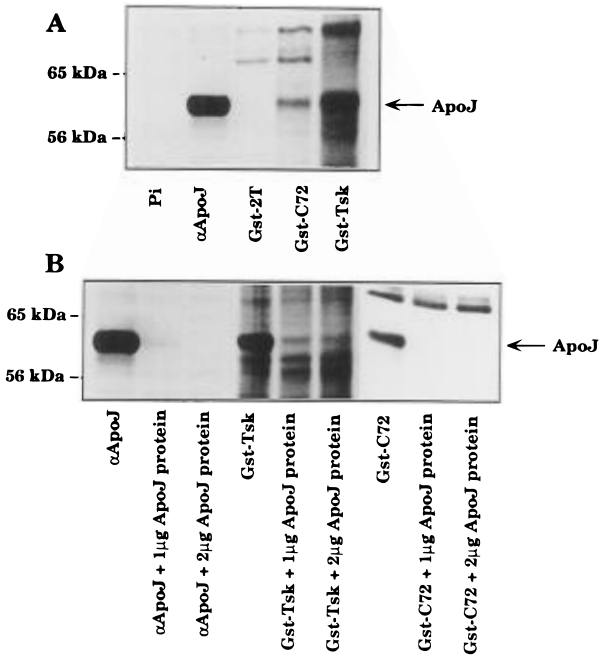

FIGURE 5: ApoJ interacts with TGFβ-receptors in vivo. Rapidly proliferating HepG2 cells were starved in methionine-deficient medium for 1 h and metabolically labeled with [^{35}S]methionine for 25 min in the same medium. Panel A: Cell lysates were incubated with anti-apoJ antibodies (α apoJ) or with various Gst-proteins (Gst-2T, control; Gst-C72, RII; Gst-Tsk7L, RI). Precipitated proteins were analyzed by SDS−PAGE under reducing conditions followed by fluorography. Panel B: Immunoprecipitations were performed in the presence of 1 and 2 μg of competing, unlabeled apoJ.

precipitation studies from metabolically labeled HepG2 cells with Gst-fusion proteins of the RI and RII receptors. HepG2 cells were pulsed for 25 min with [^{35}S]methionine to label endogenous apoJ, which has an intracellular molecular mass of approximately 60 kDa under reducing conditions (Burkey et al., 1991). The cell lysates were then subjected to precipitation either with anti-apoJ or with RI and RII Gst-beads. The results shown in Figure 5A demonstrate that TGFβ receptor Gst-beads (Gst-C72 and Gst-Tsk), but not Gst-2T control beads, were able to precipitate labeled intracellular apoJ from cellular lysates. The precipitated band with a molecular weight of 60 kDa comigrated with immunoprecipitated apoJ, and represents the intracellular, noncleaved form of apoJ. Unlabeled apoJ protein can compete-out the ^{35}S-labeled apoJ band precipitated either with anti-apoJ antibodies or with Gst-Tsk (RI) and C72 (RII) beads (Figure 5B), demonstrating the specificity of this binding.

In this report, using the intracellular domain of the RII receptor as "bait" in the yeast two-hybrid system, we identified apoJ/clusterin as a receptor-associated protein. We show that apoJ present in plasma, purified apoJ, or metabolically labeled intracellular apoJ binds to both the RI and RII receptors. The significance of this interaction is unknown. ApoJ/clusterin is highly conserved and ubiquitously expressed, but its precise physiological function is unclear. It has been proposed to have roles in complement regulation (Jenne & Tischopp, 1989), sperm maturation (Sylvester et al., 1984), lipid transport (Burkey et al., 1992), neurodegenerative diseases (May et al., 1990), epithelial cell differentiation (Hartmann et al., 1991), apoptosis (Buttyan et al., 1989), and in *src*-induced transformation (Michel et al., 1989). Apo J is expressed as a 56−60 kDa precursor which undergoes conversion to a 75−80 kDa precursor through N-linked glycosylation. This 80 kDa form of the protein undergoes intracellular cleavage to yield α and β subunits which form a disulfide-linked heterodimer which ultimately gets secreted (Burkey et al., 1991). However, recent results suggest that an intracellular form of the protein exists and may be responsible for mediating some of the biological responses attributed to apoJ. In smooth muscle cells, apoJ expression was demonstrated intracellularly and postulated to be involved in their differentiation to the nodular form (Thomas-Salgar & Millis, 1994). Expression of the protein has been shown to correlate with the induction of apoptosis in several tissues, and, interestingly, a nuclear form of clusterin has been alluded to as analyzed by immunofluorescence studies in MCF-7 cells treated with vitamin D3 (Wilson et al., 1995). In a mouse mammary carcinoma model, progression to the androgen-independent state is also proposed to correlate with what is thought to be a subcellular relocalization of clusterin from the cell membrane to the nucleus (Wilson et al., 1995).

Recent data from our laboratory have demonstrated that an intracellular form of apoJ exists in epithelial cells, and that accumulation of this form of the protein in the nucleus is induced by TGFβ (personal communication; Reddy et al., 1995). We demonstrate *in vitro* that apoJ protein can be translated from two in-frame ATG sites. Initiation from the first site encodes for the secretory form of apoJ, and initiation from the second ATG, located 33 amino acids downstream of the first and lacking the hydrophobic signal sequence, encodes for a truncated apoJ protein. Interestingly, both of these apoJ forms contain an SV-40-like nuclear localization sequence (NLS) from amino acid 52 to 58. In the truncated species of apoJ, lacking the signal sequence, presumably the NLS is dominant and the protein is targeted to the nucleus. We further show that this truncated form of apoJ translated *in vitro* is identical to that isolated from TGFβ-treated epithelial cells as analyzed by V8-protease analysis (personal communication; Reddy et al., 1995).

It has been shown that apoJ can associate with members of the Kex2-related protease family, important in neuroendocrine prohormone processing in secretory chromaffin granules of adrenal medullary cells, and it has been suggested that apoJ is involved in targeting these proteases to the granule (Palmer & Christie, 1992). Perhaps an intracellular form of apoJ similarly serves as a chaperone in the processing and trafficking of the TGFβ receptor. Other serine/threonine kinases and receptors require chaperones for correct trafficking. For example, Raf requires Hsp90 (Wartmann & Davis, 1994) and the T-cell receptor oligomeric complex requires calnexin (Hochstenbach et al., 1992) for proper assembly and transport. Interestingly, these proteins share with the recently isolated RI receptor-associated protein FKBP-12 (Wang et al., 1994) an enzymatic activity, peptidyl-proline *cis-trans* isomerase, common among most molecular chaperones.

To date, all TGFβ receptor-associated proteins are with the RI-TGFβ receptor (Wang et al., 1994; Kawabata et al., 1995; Liu et al., 1995). ApoJ/clusterin is the first example of an RII-associated protein, and it can interact with both RI and RII. Although the significance of the TGFβ receptor−apoJ interaction is not known, a link between the two has previously been established. Similarities in the expression pattern of the genes encoding TGFβ, TGFβ receptors, and apoJ have been demonstrated during mouse

embryogenesis (French et al., 1993), epithelial cell differentiation (Hartmann et al., 1991), and cardiac valve morphogenesis (Witte et al., 1994). Both apoJ and TGFβ are stored in platelets and released during platelet activation at sites of tissue injury (Witte et al., 1993), and both are elevated at sites of injury in fibrotic lesions (Buttyan et al., 1989). In addition, TGFβ has been shown to modulate the expression of apoJ in a variety of cell types including smooth muscle cells (Thomas-Salgar & Millis, 1994) and mammary epithelial cells (Tenniswood et al., 1992). Further experiments will be required to determine the precise cellular function of apoJ and the significance of its interaction with the TGFβ receptors.

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