Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6997–7001, July 1994
Biochemistry

Mapping of the ligand binding domain of the transforming growth factor β receptor type III by deletion mutagenesis

(betaglycan)

MARIE-CLAUDE PEPIN, MICHEL BEAUCHEMIN, JOSÉE PLAMONDON, AND MAUREEN D. O'CONNOR-MCCOURT*

Cell Surface Recognition Group, Biotechnology Research Institute, National Research Council Canada, Montreal, PQ Canada H4P 2R2

Communicated by Harvey F. Lodish, March 11, 1994

ABSTRACT **Transforming growth factor β (TGF-β) receptor type III is a membrane-anchored proteoglycan that binds TGF-β via the core protein. We have determined, by deletion mutagenesis of the receptor type III, the minimal essential region of the extracellular domain that is capable of binding TGF-β. Nine deletion mutants were produced, six of which are expressed on the cell surface and bind TGF-β. We find that the shortest of these active mutants, which retains only 253 of the 785 amino acids of the extracellular domain, binds TGF-β with the same affinity as the full-length receptor. These results indicate that the ligand binding domain lies proximal to the transmembrane domain and is functionally independent from the rest of the extracellular domain. We have determined from the mutants that one of the potential glycosaminoglycan attachment sites in the receptor type III is not utilized. Results from the nonglycosylated mutants confirm that the glycosaminoglycan chains are not required for the folding, targeting, and TGF-β binding activity of the receptor. Moreover, we present evidence for dimerization and multimerization of the receptor.**

Transforming growth factors β [TGF-βs (TGF-β1–TGF-β3 in mammals)] are closely related secreted peptides that regulate extracellular matrix formation, cell growth, and differentiation (1, 2). Most cells display three types of cell surface TGF-β receptors that can be divided into two groups (3, 4): those thought to be involved in signaling (receptor types I and II) (5) and receptor type III, which is thought to act as an enhancer of TGF-β access to the signaling receptors (6, 7).

Receptor types I and II seem to be directly involved in signal transduction since the loss of expression of either type I or types I and II receptors results in the loss of response to the growth-inhibitory effect of TGF-β in tumor cell lines (8–12) or in mutagenized mink lung epithelial cells (13–16). Recent cloning of type I and type II receptors revealed that their cytoplasmic domains contain serine/threonine kinase motifs suggesting that they mediate a phosphorylation-dependent transduction of signal (5, 17–19). Receptor type I is a 53-kDa transmembrane protein that requires the presence of receptor type II in order to bind TGF-β (5, 16, 19). On the other hand, receptor type II is a 73-kDa protein that can independently bind TGF-β (17) but requires the presence of receptor type I for signaling (5).

Receptor type III is coexpressed with receptor types I and II in various cell types and in most cases is the major TGF-β binding molecule on the cell surface (1, 9, 20, 21). Receptor type III exists as a proteoglycan with heparin and chondroitin sulfate chains; however, TGF-β can bind to the core of the receptor in the absence of glycosaminoglycan (GAG) chains (22, 23). Cloning of receptor type III showed that it is a transmembrane protein with a short cytoplasmic domain showing no apparent signaling motif (24–26). The absence of an obvious signaling domain, together with the fact that various TGF-β responsive cells lack the type III receptor (9, 27, 28), has led to the proposal that the type III receptor does not participate directly in TGF-β signal transduction. Receptor types I and II bind TGF-β1 and TGF-β3 with higher affinity than TGF-β2 (9, 29, 30) and this difference is more pronounced in cells that lack receptor type III (27, 30). This led to the suggestion that receptor type III might modify the responsiveness of cells to various isoforms of TGF-β. More recently, results that demonstrated a direct association between the type III and signaling receptors provided strong support for the role of receptor type III in delivering TGF-β to the signaling receptors (6, 7).

In this work, we have performed an analysis of the functional subdomains of the extracellular domain of receptor type III focusing on the TGF-β-binding function. We have produced deletion mutants of receptor type III in order to localize the amino acids critical for TGF-β binding. We find that mutants lacking up to three-quarters of the N-terminal portion of receptor type III can still bind TGF-β, indicating that the binding site is located close to the transmembrane domain.

EXPERIMENTAL PROCEDURES

Production of Deletion Mutants. A *Hin*dIII cDNA fragment containing the complete coding region of receptor type III was subcloned from clone R3-OFF (25) into pTZ19R (Pharmacia LKB) at the single *Hin*dIII site to produce pTZ19R-RTB3. Deletion mutants were produced by progressive digestion of pTZ19R-RTB3, with BAL-31 exonuclease, starting from a *Stu* I site located in the receptor type III coding region, 20 amino acids downstream from the signal peptide. BAL-31 digests were repaired with the Klenow fragment of DNA polymerase I and digested with *Kpn* I to isolate BAL-31/*Kpn* I fragments containing the 3′ portion of the coding region. These fragments were subcloned into pTZ19R-RTB3 digested with *Stu* I and *Kpn* I (unique sites), thereby joining the internally deleted coding region to the first 40 amino acids of the receptor (signal peptide plus the 20 first amino acids). Clones harboring in-frame deletions were identified by nucleotide sequence determination of the *Stu* I–BAL-31 junction. The coding region of these mutants (contained in a *Hin*dIII fragment) was subcloned into the *Hin*dIII-digested pcDNAIneo expression vector (Invitrogen).

Cell Culture and Transfection Procedure. COS-1 cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum. Cells were transiently transfected according to the procedure of Chen and Okayama (31). Transfections were performed using 20 μg of expression

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TGF-β, transforming growth factor β; GAG, glycosaminoglycan.

*To whom reprint requests should be addressed at: Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, PQ Canada H4P 2R2.

vector per six-well plate. Functional binding of TGF-β to the mutant receptors was monitored 48 hr after transfection by a ligand binding and cross-linking assay.

Binding Assay. Binding and cross-linking assays were performed as described (32) using TGF-β1 (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle) or TGF-β2 (Celtrix Laboratories, Palo Alto, CA), which was iodinated as described (33). Cross-linked receptors were analyzed by PAGE (under reducing or nonreducing conditions) and autoradiography.

Enzymatic Deglycosylation of Affinity-Labeled COS Transfected Cells. Enzymatic removal of the GAG chains with heparitinase (ICN) and chondroitinase ABC (Seikagaku America, Rockville, MD) was performed according to Cheifetz *et al.* (23).

RESULTS

The experimental strategy that we designed for localizing the TGF-β binding domain of receptor type III relied in part on experimental evidence from our laboratory that suggested that the ligand binding site may lie within the C-terminal portion of the extracellular domain. We have observed two bands on Bewo choriocarcinoma cells that are affinity labeled with TGF-β. The first is a diffuse 400- to 150-kDa band that is characteristic of receptor type III. The other is a 50-kDa band that, due to its binding characteristics, was suggested by us to be a proteolytic fragment of receptor type III (34). We have now observed that both bands are specifically immunoprecipitated by an anti-peptide antibody directed against the C-terminal cytoplasmic domain of receptor type III (not shown). This result indicates that the 50-kDa band is a proteolytic fragment of receptor type III and that it contains both the cross-linked ligand and the cytoplasmic domain. Due to the small size of this fragment, the portion of the extracellular domain that becomes affinity labeled with ^{125}I-labeled TGF-β (^{125}I-TGF-β) must be proximal to the transmembrane domain. Since we cannot ascertain if the proteolytic event has occurred before or after binding of TGF-β, we cannot conclude from these results whether this 50-kDa fragment can independently bind TGF-β or whether it is a degradation product of the previously labeled intact receptor. Similarly, Cheifetz *et al.* (23) have shown that a 63- to 72-kDa affinity-labeled trypsin fragment of receptor type III remains on the cell surface, suggesting that the binding site is close to the transmembrane domain. We undertook to delineate the minimal region of receptor type III that is capable of binding TGF-β by producing a set of progressive deletion mutants starting from the N-terminal end of the molecule. We obtained nine in-frame deletion mutants (Fig. 1) of TGF-β receptor type III that lack between 261 and 581 amino acids of the N-terminal portion of the extracellular domain.

Affinity-Labeling of Deletion Mutants. We assayed the binding ability of the mutant receptors by affinity-labeling (with iodinated TGF-β2) COS-1 cells that were transiently transfected with expression vectors for the full-length receptor type III or the deletion mutants. The labeled receptor bands were analyzed on nonreducing SDS/PAGE (Fig. 2). Results of the affinity-labeling assays show that mutants lacking up to 532 amino acids of the N-terminal portion of receptor type III are expressed on the cell surface and can still bind TGF-β. This labeling was blocked by a 10-fold molar excess of nonradioactive TGF-β, indicating that the binding was specific (see Fig. 4 and data not shown). Similar results were obtained when the mutants were labeled with ^{125}I-TGF-β1 (not shown).

With the wild-type receptor, the specifically labeled bands appear as a diffuse band in the 140- to 400-kDa region and a sharp band at 115 kDa. The smear is characteristic of receptor type III linked to GAG chains, whereas the sharp 115-kDa band

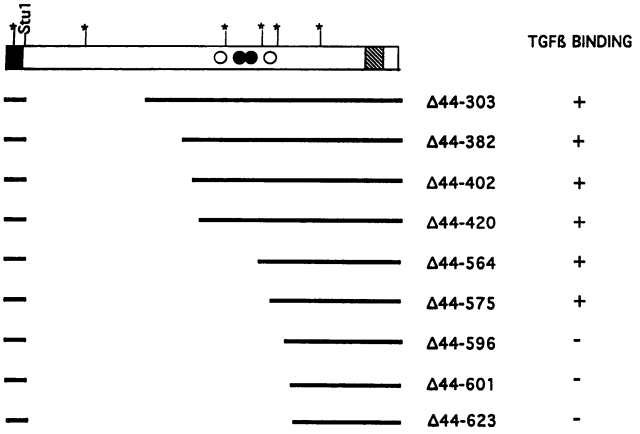

FIG. 1. Schematic representation of TGF-β receptor type III deletion mutants. TGF-β receptor type III is represented at the top. ■, Signal peptide; □, transmembrane domain. Potential proteoglycan attachment sites are indicated by circles (most favorable are filled circles); the potential N-linked glycosylation sites are indicated by stars. Mutants are named by the range of amino acids deleted. The TGF-β binding ability as determined in Figs. 2 and 3 is summarized on the righthand side.

is characteristic of the core protein of receptor type III. Cells that were transfected with the parental vector [pcDNAIneo (Fig. 2, vector)] yielded a similar affinity-labeling pattern, albeit very faint. This faint labeling results from the presence of endogenous receptor type III in the COS-1 cells.

The labeling pattern observed with mutants Δ44–303, Δ44–382, Δ44–402, and Δ44–420 is qualitatively similar to that of the wild-type receptor—i.e., both the diffuse band of high molecular mass (corresponding to the glycosylated proteoglycan form of the receptor) and the sharp band of lower molecular mass (corresponding to the core protein) are observed. Molecular masses of the core proteins of Δ44–303, Δ44–382, Δ44–402, and Δ44–420 were determined from the gel to be 60 kDa, 55 kDa, 50 kDa, and 49 kDa, respectively, which correspond to those predicted from the sequence (see Table 1). Different results were obtained with mutants Δ44–564 and Δ44–575. With these two mutants, we found that the diffuse band that is characteristic of the proteoglycan form of receptor type III is lost, and instead a set of four labeled bands is observed. The change in the electrophoretic pattern of these two mutants likely represents the loss of the GAG attachment site(s). In fact, three potential GAG attachment sites exist in the region differentiating Δ44–564 from Δ44–420 (Fig. 1). The bands marked by filled arrowheads (Fig. 2) represent the unmodified core protein of these mutants since their molecular masses correspond to that predicted from the sequence (33 kDa for Δ44–564 and 29 kDa for Δ44–575). The presence of the three other bands (Fig. 2, open arrowheads) is consistent with the existence of di-, tri-, and tetrameric forms of the receptor—that is, the molecular masses of the four bands seen with mutants Δ44–564 and Δ44–575 are roughly multiples of the molecular mass of the core protein (see Table 1 and discussion below). The identity of the strongly labeled band that lies just below the core of Δ44–575 is uncertain at present. It may represent a degradation product. No affinity-labeled bands were observed when COS-1 cells were transfected with Δ44–623 (Figs. 2 and 3) or Δ44–596 or Δ44–601 (Fig. 3).

Deglycosylation Analysis. To further our analysis, we enzymatically removed the GAG chains from the various affinity-labeled mutants and wild-type receptor as described by Cheifetz *et al.* (23) and analyzed the samples on reducing SDS/PAGE. As expected, treatment of the extract from cells transfected with the wild-type receptor with chondroitinase and heparitinase resulted in the disappearance of the char-

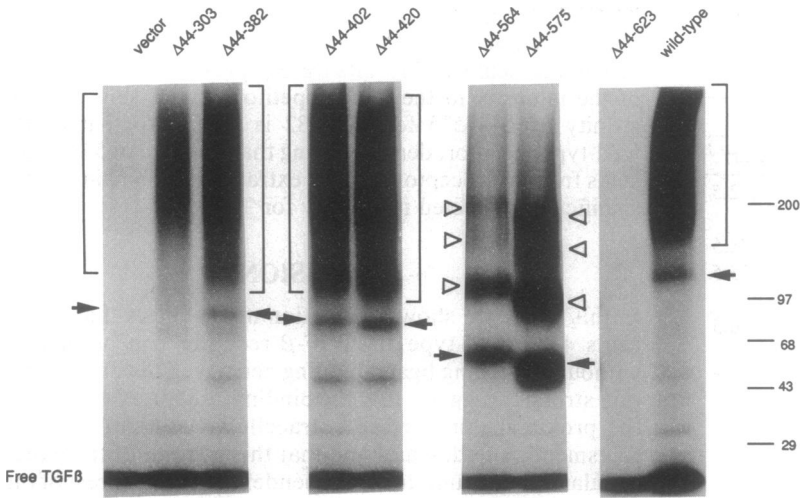

FIG. 2. Affinity-labeling of TGF-β receptor type III deletion mutants. Affinity-labeling with ^{125}I-TGF-β2 was performed as described (33) on COS-1 cells that were transiently transfected with expression vector for the full-length receptor type III (wild-type), the N-terminal deletion mutants of receptor type III, or the pcDNAIneo vector (vector). Cell extracts were submitted to SDS/PAGE on a 3–12% polyacrylamide gel gradient under nonreducing conditions. Molecular mass markers are indicated in kDa. Brackets delineate the high molecular mass smear characteristic of the proteoglycan form of the receptor. Filled arrowheads point to the core protein of the receptor and mutants. Open arrowheads for mutant Δ44–564 and Δ44–575 point to discrete labeled species of higher molecular mass that are likely to be multimers of the core protein (see text). The smaller band corresponds to free TGF-β.

acteristic 400- to 140-kDa diffuse band and a concomitant increase in the intensity of the 115-kDa core protein (Fig. 3, filled arrowhead). Interestingly, a faint chondroitinase- and heparitinase-resistant doublet with an apparent molecular mass of ≈258 kDa is also present in the treated extract (Fig. 3, open arrowhead). This doublet is roughly double the size of the core protein, supporting the proposed existence of a dimer of the core.

Similar affinity-labeling patterns after enzymatic treatment were observed for mutants Δ44–382, Δ44–402, and Δ44–420, thereby confirming the size of the core proteins and supporting the occurrence of dimerization in all of these cases. For mutant Δ44–303, enzymatic treatment resulted in the disappearance of the diffuse band and the appearance of multiple bands of unclear identity. This may indicate that Δ44–303 is more susceptible to proteolysis than the other mutants.

Enzymatic treatment of labeled Δ44–564 and Δ44–575 did not alter their electrophoretic pattern, confirming the absence of removable GAG chains on these two mutants. Here again, bands with double the molecular mass of the core can be observed, as well as bands with higher molecular masses suggesting that they are oligomers of the core. The fact that these oligomers were observed on both reducing (Fig. 3) and nonreducing (Fig. 2) SDS/PAGE indicates that they are not linked by disulfide bonds. Because of their molecular mass, the labeled species seen with Δ44–596 and Δ44–601 likely represents the endogenous receptor type III from COS cells.

Molecular Mass Analysis. The molecular masses of the various labeled species present in Figs. 2 and 3 are compiled in Table 1. The apparent molecular masses of the core proteins measured from Fig. 2 were corrected by subtracting the molecular mass of the cross-linked TGF-β2 dimer (25 kDa) since the electrophoresis was performed under nonreducing conditions. These corrected molecular masses correspond very closely to those predicted from the sequences. The molecular masses of the core proteins measured from Fig. 3 were corrected by subtracting the molecular mass of the cross-linked TGF-β2 monomer (12.5 kDa) since the electrophoresis was performed under reducing conditions. The results show that the corrected molecular masses of all core proteins were 18–20 kDa larger than that predicted from the sequence. The change in molecular mass between mutants, however, is consistent with the deletions introduced. The reason for this overestimation of the molecular mass under reducing conditions is unclear. However, it may be due to the presence of N-linked glycosylations since our shortest binding mutant (Δ44–575) still retains two of the six potential N-glycosylation sites of the full-length receptor type III.

Analysis of the relationship between the multiple bands seen for mutants Δ44–564 and Δ44–575 strongly suggests that

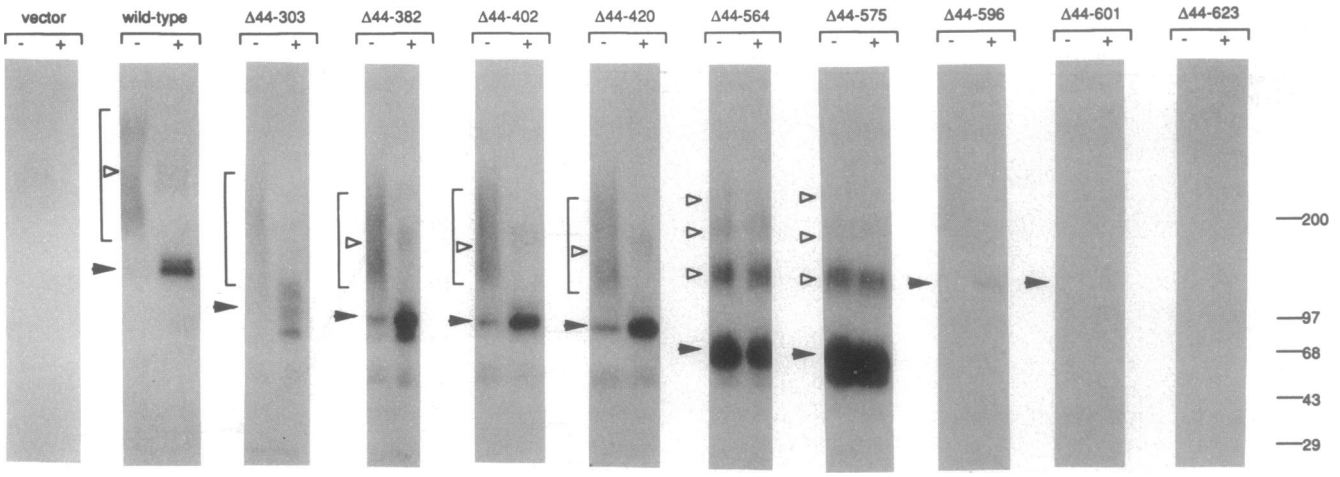

FIG. 3. Enzymatic removal of GAG chains from the affinity-labeled receptor type III and deletion mutants. COS-1 cells were transfected with the pcDNAIneo vector (vector), the pcDNAIneo-RTB3 receptor type III expression vector (wild-type), or the various deletion mutants. Following binding and cross-linking with ^{125}I-TGF-β2, samples were either untreated (−) or subjected to enzymatic digestion with chondroitinase and heparitinase (+) prior to SDS/PAGE analysis under reducing conditions. Brackets point to the proteoglycan form of the receptor and mutants. Filled arrowheads point to the core protein of the receptor or mutants. Open arrowheads point to labeled species of higher apparent molecular mass, possibly dimers and multimers (see text). Molecular mass markers are indicated in kDa.

Table 1. Molecular masses of the wild-type and mutant receptors as determined from Figs. 2 and 3

	Molecular mass, kDa				
		Fig. 2		Fig. 3	
Receptor	Pred.*	Meas.	Corr.†	Meas.	Corr.‡
Wild-type	94.1	115	90	125 258§	112.5
Δ44–303	62.7	85	60	91	78.5
Δ44–382	53.5	80	55	86 163§	73.5
Δ44–402	51.5	75	50	83 151§	70.5
Δ44–420	49.4	74.7	49	80 149§	67.5
Δ44–564	33.9	58 98§ 146§ 198§	33	66 129§ 188§ 258§	53.5
Δ44–575	32.8	54 90§ 133§ 180§	29	64 129§ 188§	51.5

Pred., predicted; Meas., measured; Corr., corrected.
*Predicted from sequence analysis.
†Corrected by substracting the molecular mass of the cross-linked TGF-β2 dimer (nonreducing conditions).
‡Corrected by substracting the molecular mass of the cross-linked TGF-β2 monomer (reducing conditions).
§Labeled species of higher molecular mass than the core protein.

these are oligomers of the core protein. Under reducing conditions (Fig. 3), where the disulfide bond between the two halves of the TGF-β2 dimer is broken, molecular masses of the labeled bands correspond to multiples of the apparent molecular mass of the core protein plus TGF-β monomer. For Δ44–564, the core plus TGF-β monomer has a molecular mass of 66 kDa, whereas the other bands have molecular masses of 129 kDa (≈2 × 66), 188 kDa (≈3 × 66), and 258 kDa (≈4 × 66). A similar result is obtained with Δ44–575.

Affinity-Labeling Competition. We compared the affinity of the shortest binding mutant (Δ44–575) for TGF-β2 to that of the wild-type receptor by carrying out affinity-labeling competition studies. Cells were transfected with either expression vector and affinity-labeling was performed with ^{125}I-TGF-β2 (0.2 nM) in the presence of increasing amounts of nonradioactive TGF-β2. Band intensities were quantitated densitometrically and results were expressed as a percentage of the intensity in the noncompetitor extract (Fig. 4). The affinity of Δ44–575 for TGF-β2 is similar to that of the wild-type receptor, demonstrating that removal of 532 amino acids from the receptor type III extracellular domain has not significantly affected its affinity for TGF-β2.

DISCUSSION

In this paper we show by deletion mutagenesis that amino acids 44–575 of type III TGF-β receptor can be deleted without abolishing ligand binding activity. This demonstration strongly suggests that the binding site for TGF-β lies in the proximal third of the extracellular domain, near the transmembrane domain, and that this portion of the extracellular domain functions independently from the rest of the extracellular domain. At present, we cannot formally exclude the possibility that the first 20 amino acids of the receptor also contribute to the binding function since, owing to the experimental strategy, they are present in all of the mutants. The conclusion that the ligand binding site is close to the transmembrane domain is consistent with previous results where we observed that a TGF-β-labeled 50-kDa receptor type III fragment is immunoprecipitated with an antibody against the cytoplasmic C-terminal portion of the receptor. We also demonstrated that the affinity of the shortest binding mutant is indistinguishable from that of the wild-type receptor. This indicates that this mutant will provide a good model for the further definition of the ligand binding site of receptor type III.

We have also shown that mutant Δ44–596 (and shorter ones) cannot bind TGF-β. It is likely that the 21 amino acids that are missing from Δ44–596 but present in Δ44–575 are important for the ligand binding activity. Alternatively, it may be that these amino acids are necessary for the proper folding, transport, and/or stability of the mutants.

TGF-β receptor type III shows homology to endoglin (35). The degree of homology is highest in the transmembrane domain and cytoplasmic domain (71% identity). The extracellular domains of these proteins show limited similarity, a result that is interesting in light of the fact that both can bind TGF-β (21). It is also interesting to note that the two regions in the extracellular domain that show 48% (amino acids

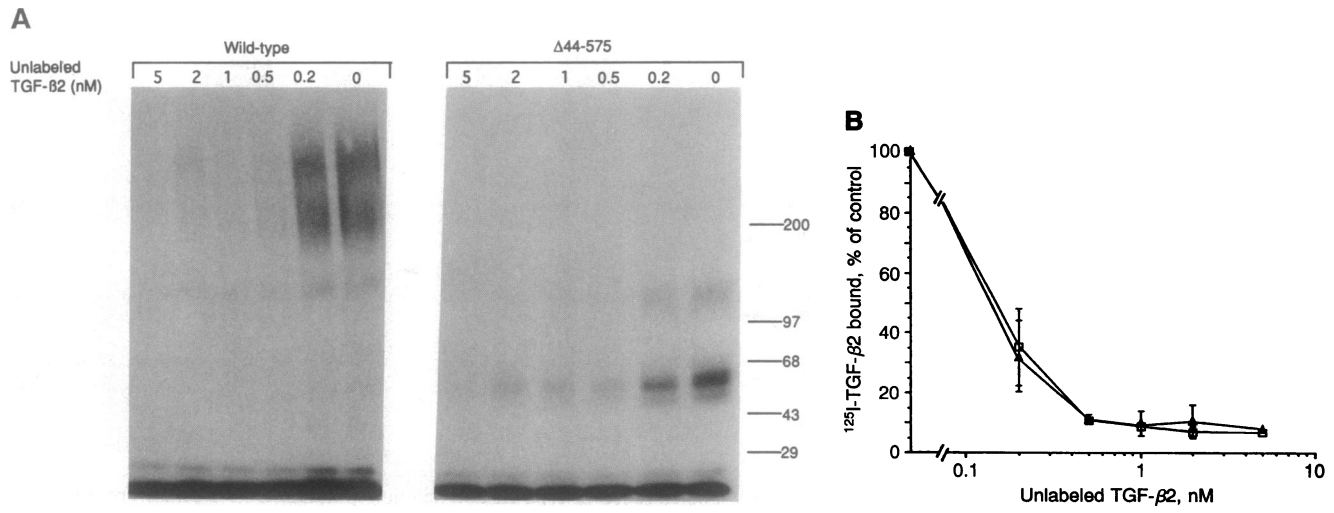

FIG. 4. Comparison of the relative affinities of TGF-β receptor type III and the Δ44–575 mutant for TGF-β2. (*A*) COS-1 cells transfected with either pcDNAIneo-RTβ-III or Δ44–575 were labeled with ^{125}I-TGF-β2 (0.2 nM) in the presence of increasing amounts of competitor unlabeled TGF-β2. Signal intensities were quantified using a LKB 2400 GelScan XL. Molecular mass markers are indicated in kDa. (*B*) Results are expressed as the percentage of binding as a function of the concentration of unlabeled competitor.

453–513) and 28% (amino acids 18–298) homology between endoglin and receptor type III can be deleted without affecting binding. In the region retained in the shortest binding-effective mutant (Δ44–575), we note a number of short stretches of homology between endoglin and receptor type III that could point to critical residues involved in the binding function. The minimal ligand binding domain of receptor type III lies within a region of homology between TGF-β receptor type III, uromodulin, glycoprotein GP-2, and sperm receptors Zp2 and Zp3. It was proposed that these conserved domains might serve analogous functions (36). Our results support the idea that these are ligand binding functions. The fact that the cysteine residues, but not the aromatic or hydrophobic residues, are invariant within these domains suggests that the three-dimensional structure of the ligand binding domain is conserved while the residues that form the contact sites vary. It is not surprising then that these proteins exhibit a common ligand binding function yet are able to interact with very different ligands [i.e., 95-kDa sperm proteins for Zp2 and Zp3, mannose-sensitive fimbriated microorganisms for uromodulin, and TGF-β for the receptor type III (36)].

During the preparation of this manuscript a similar conclusion in relation to the location of the binding site within receptor type III was made by Fukushima *et al.* (37) using an alternative strategy. They expressed in bacteria various portions of the extracellular domain of receptor type III and tested the binding and functional activity of these polypeptides after purification. The only TGF-β binding peptide that they identified (Bg3) contains receptor type III amino acids 543–769. This is similar in length to our Δ44–575 mutant. The affinity of the Bg3 peptide for TGF-β1 is comparable to that of the wild-type receptor. We similarly found that the affinity of Δ44–575 for TGF-β2 is comparable to that of the wild-type receptor.

Comparison of mutants Δ44–420 and Δ44–564 indicates that deletion of amino acids 420–564 results in the loss of the GAG modifications on the receptor, suggesting that the GAG attachment site(s) is located between amino acids 420 and 564. Indeed, three potential GAG attachment sites have been localized to this area (serines 477, 535, and 546) by sequence analysis (24, 25). The fact that mutants Δ44–564 and Δ44–575 do not bear GAG chains indicates that the potential GAG chain attachment site at amino acid 582 is not used.

We observed that mutants Δ44–564 and Δ44–575, which have the GAG chain attachment sites removed, show a pattern of four bands labeled with TGF-β. As previously stated, the apparent molecular mass of these species is consistent with them being monomer and oligomers of the mutants. Enzymatic removal of the GAG chains from the wild-type receptor and glycosylated mutants also allowed visualization of labeled species with apparent molecular masses double that of the core protein. Since we have affinity-labeled the receptor in all of our experiments, it cannot be stated whether these dimers and oligomers exist in the absence of ligand or are brought about by the binding of TGF-β. It is likely, however, that binding of TGF-β (which is a dimer itself) induces receptor dimerization either by bridging the receptor molecules or by inducing a conformational change in the receptor. These dimers would then be covalently trapped by the cross-linking agent. We have not been able to identify mutants that still bind TGF-β and are unable to oligomerize. This indicates that the receptor dimerization/oligomerization property colocalizes with the ligand binding function and suggests that TGF-β may be bridging the receptor dimers.

Receptor type III has been shown to form heterodimers with type II receptor in the presence of ligand (6, 7). Our results suggest that receptor type III is also capable of forming homooligomers. It has been proposed that the type III/type II heterodimer is formed so that receptor type III can assist type II receptor in binding TGF-β2 (6, 7). The function of receptor type III homooligomers is not known at present.

In summary, the ability to produce deletion mutants that lack up to three-quarters of the extracellular domain and still bind TGF-β suggests that the binding domain is restricted to the proximal part of the extracellular domain and that this domain is folded and expressed relatively independently from the remainder of the extracellular domain.

We thank Herbert Lin for supplying the cDNA for receptor type III and Aris Moustakas for supplying the anti-peptide antibody directed against the C-terminal cytoplasmic domain of receptor type III. This is National Research Council Canada publication no. 36831.

1. Massagué, J. (1990) *Annu. Rev. Cell Biol.* **6**, 18586–18592.
2. Sporn, M. B. & Roberts, A. B. (1992) *J. Cell Biol.* **119**, 1017–1021.
3. Massagué, J. (1992) *Cell* **69**, 1067–1070.
4. Lin, H. Y. & Lodish, H. F. (1993) *Trends Cell Biol.* **3**, 14–19.
5. Wrana, J. L., Attisano, L., Cárcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F. & Massagué, J. (1992) *Cell* **71**, 1003–1014.
6. López-Casillas, F., Wrana, J. L. & Massagué, J. (1993) *Cell* **73**, 1435–1444.
7. Moustakas, A., Lin, H. Y., Henis, Y. I., Plamondon, J., O'Connor-McCourt, M. & Lodish, H. F. (1993) *J. Biol. Chem.* **268**, 22215–22218.
8. Kimchi, A., Wang, X. F., Weinberg, R. A., Cheifetz, S. & Massagué, J. (1988) *Science* **240**, 196–199.
9. Segarini, P., Rosen, D. M. & Seyedin, S. M. (1989) *Mol. Endocrinol.* **3**, 261–272.
10. Massagué, J., Cheifetz, S., Boyd, F. & Andres, J. (1990) *Ann. N.Y. Acad. Sci.* **593**, 59–72.
11. Siepl, C., Malipiero, U. V. & Fontana, A. (1991) *J. Immunol.* **146**, 3063–3067.
12. Geiser, A. G., Burmester, J. K., Webbink, R., Roberts, A. B. & Sporn, M. B. (1992) *J. Biol. Chem.* **267**, 2588–2593.
13. Chinkers, M. (1987) *J. Cell. Physiol.* **131**, 1–5.
14. Boyd, F. T. & Massagué, J. (1989) *J. Biol. Chem.* **264**, 2272–2278.
15. Laiho, M., Weis, M. B. & Massagué, J. (1990) *J. Biol. Chem.* **265**, 18518–18524.
16. Laiho, M., Weis, F. M., Boyd, F. T., Ignotz, R. A. & Massagué, J. (1991) *J. Biol. Chem.* **266**, 9108–9112.
17. Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A. & Lodish, H. F. (1992) *Cell* **68**, 775–785, and erratum (1992) **70**, 1069.
18. Tsuchida, K., Lewis, A. K., Mathews, L. & Vale, W. W. (1993) *Biochem. Biophys. Res. Commun.* **191**, 790–795.
19. Ebner, R., Chen, R.-H., Shum, L., Lawler, S., Zioncheck, T., Lee, A., Lopez, A. & Derynk, R. (1993) *Science* **260**, 1344–1348.
20. Cheifetz, S., Like, B. & Massagué, J. (1986) *J. Biol. Chem.* **261**, 9972–9978.
21. Cheifetz, S., Bellón, T., Calés, C., Vera, S., Bernabeu, C., Massagué, J. & Letarte, M. (1992) *J. Biol. Chem.* **267**, 19027–19030.
22. Segarini, P. & Seyedin, S. M. (1988) *J. Biol. Chem.* **263**, 8366–8370.
23. Cheifetz, S., Andres, J. L. & Massagué, J. (1988) *J. Biol. Chem.* **263**, 16984–16991.
24. López-Casillas, F., Cheifetz, S., Doddy, J., Andres, J. L., Lane, E. L. & Massagué, J. (1991) *Cell* **67**, 785–795.
25. Wang, X.-F., Lin, H. Y., Ng-Eaton, E., Downward, J., Lodish, H. F. & Weinberg, R. A. (1991) *Cell* **67**, 797–805.
26. Morén, A., Echijo, H. & Miyazono, K. (1992) *Biochem. Biophys. Res. Commun.* **189**, 356–362.
27. Ohta, M., Greenberger, J. S., Anklesaria, P., Bassols, A. & Massagué, J. (1987) *Nature (London)* **329**, 539–541.
28. Massagué, J., Cheifetz, S., Endo, T. & Nadal-Ginard, B. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8206–8210.
29. Cheifetz, S., Weatherbee, J. A., Tsang, M. L.-S., Anderson, J. K., Mole, J. E., Lucas, R. & Massagué, J. (1987) *Cell* **48**, 409–415.
30. Cheifetz, S., Hernandez, H., Laiho, M., tenDijke, P., Iwata, K. K. & Massagué, J. (1990) *J. Biol. Chem.* **265**, 20533–20538.
31. Chen, C. & Okayama, H. (1987) *Mol. Cell. Biol.* **7**, 2745–2752.
32. Mitchell, E. J. & O'Connor-McCourt, M. (1991) *Biochemistry* **30**, 4350–4356.
33. Philip, A. & O'Connor-McCourt, M. (1991) *J. Biol. Chem.* **266**, 22290–22296.
34. Mitchell, E. J., Lee, K. & O'Connor-McCourt, M. (1992) *Mol. Biol. Cell* **3**, 1295–1307.
35. Gougos, A. & Letarte, M. (1990) *J. Biol. Chem.* **265**, 8361–8364.
36. Bork, P. & Sander, C. (1992) *FEBS Lett.* **300**, 237–240.
37. Fukushima, D., Bützow, R., Hildebrand, A. & Ruoslahti, E. (1993) *J. Biol. Chem.* **268**, 22710–22715.