Cloning of a Type I TGF-β Receptor and Its Effect on TGF-β Binding to the Type II Receptor

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Transforming growth factor–β (TGF-β) affects cellular proliferation, differentiation, and interaction with the extracellular matrix primarily through interaction with the type I and type II TGF-β receptors. The type II receptors for TGF-β and activin contain putative serine-threonine kinase domains. A murine serine-threonine kinase receptor, Tsk 7L, was cloned that shared a conserved extracellular domain with the type II TGF-β receptor. Overexpression of Tsk 7L alone did not increase cell surface binding of TGF-β, but coexpression with the type II TGF-β receptor caused TGF-β to bind to Tsk 7L, which had the size of the type I TGF-β receptor. Overexpression of Tsk 7L inhibited binding of TGF-β to the type II receptor in a dominant negative fashion. Combinatorial interactions and stoichiometric ratios between the type I and II receptors may therefore determine the extent of TGF-β binding and the resulting biological activities.

Growth factor–induced protein phosphorylation plays a key role in the signal transduction that leads to mitogenic responses (*1*). Most growth factor receptors are transmembrane tyrosine kinases or are associated with cytoplasmic tyrosine kinases. However, another class of transmembrane receptors is predicted to function as serine-threonine kinases (*2*). The type II activin receptors (*3–5*) and the type II TGF-β receptors (*6*) are the only known receptors of this family.

On the basis of their various biological activities (*7*), different TGF-β species are probably potent developmental regulators of cell proliferation and differentiation. Several types of TGF-β binding proteins have been detected at the cell surface (*8*). The type I and type II receptors, defined on the basis of the mobility of their ^{125}I–TGF-β cross-linked products in denaturing gels, probably mediate the majority of the activities of TGF-β (*9, 10*). The cloned type II receptor is predicted to function as a transmembrane serine-threonine kinase (*6, 10*) and is required for the antiproliferative activity of TGF-β, whereas the type I receptor mediates the induction of several genes involved in cell-matrix interactions (*11*). The dominant negative abrogation of the type II receptor–associated responses by an overexpressed truncated type II receptor suggests that receptor dimerization is critical for its function (*11*). In addition, a heterodimeric association between the type II and the type I receptor has been proposed (*9, 10*).

To pursue the molecular characterization of the type I TGF-β receptor, we generated a murine cell line, NMS 90, that expresses large quantities of type I receptors (*12*). Scatchard analysis revealed the presence of approximately 10^4 TGF-β1 binding sites at the cell surface (*13*). Cross-linking analysis of the cell surface receptors with ^{125}I-labeled TGF-β1 as ligand showed that most of the TGF-β receptors were, on the basis of their apparent size in gel, type I receptors and that little of the type II and type III receptors were expressed (*13*). Assuming that the type I TGF-β receptor might be a transmembrane serine-threonine kinase similar to the type II TGF-β receptor, we used the cDNA sequence of the activin receptor (*3*) to generate by polymerase chain reaction (PCR) a hybridization probe corresponding to the cytoplasmic kinase region of the activin receptor. A low stringency hybridization screen of a λgt10-based cDNA library from NMS 90 cells resulted in the isolation of related serine-threonine kinase receptors. The most abundant cDNA was named tsk 7L and full-size cDNAs were isolated from NMS 90 cell and NIH 3T3 cell cDNA libraries. The DNA sequences of these clones were identical.

The open reading frame of the tsk 7L cDNAs encodes a 509 amino acid polypeptide (Fig. 1). The start codon, located in a consensus initiation sequence context, is preceded by a 450-bp-long but presumably incomplete, extremely G-C–rich 5′ untranslated region. The 3′ untranslated region lacks the AAUAAA sequence usually preceding the polyadenylation site (*14*), and is therefore likely to be incomplete (*13*). The internal hydrophobic sequence (amino acids 124 to 146) probably constitutes a transmembrane domain, thus dividing the polypeptide into a 123–amino acid extracellular and a 363–amino acid cytoplasmic domain. The NH_2-terminal signal peptide is predicted (*15*) to be cleaved after residue 17, thus leaving a mature transmembrane polypeptide of 492 residues with a core protein size of about 55 kD. The extracellular domain contains one potential N-glycosylation site (residue 102) and a large number of cysteines reminiscent of other receptors. These cysteines are probably important in determining the correct three-dimensional structure, necessary for ligand binding. The ectodomains of the activin receptors (*3–5*) and Tsk 7L share little sequence identity, although both receptors share a similar cysteine cluster adjacent to the transmembrane domain (Fig. 2A). The sequence similarity between the extracellular domains of Tsk 7L and the type II TGF-β receptor (*6*) is more pronounced. Three cysteines are located upstream of the transmembrane domain in both receptors, and a sequence containing

Fig. 1. Deduced amino acid sequence of tsk 7L cDNA. The arrow after amino acid 17 indicates the most likely signal peptide cleavage site (*15*). The cysteines in the extracellular domain are boxed. The single N-glycosylation site is double underlined and the hydrophobic transmembrane region is boldly underlined. In the cytoplasmic domain, the two conserved glycines in the putative ATP binding site are marked with a bold dot, the serine-threonine kinase core motif is underlined with a dashed line, and the potential tyrosine kinase target site is underlined with a dotted line. Protein kinase C recognition sites are indicated by asterisks. The cDNAs were isolated and sequenced as described (*26*). This nucleotide sequence has been submitted to GenBank (accession number L15436).

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five cysteines downstream of the signal peptide (residues 32 to 63) of Tsk 7L displays significant similarity with the type II TGF-β receptor (Fig. 2, A and B). This conserved structural motif, which is absent in the activin receptors, may be spatially important in determining ligand binding specificity. Downstream of this conserved cysteine-rich domain, the type II TGF-β receptor contains a long sequence that is absent in Tsk 7L (Fig. 2B). The structural similarities of Tsk 7L and the type II TGF-β receptor possibly result in three-dimensional similarities in their ectodomains.

The cytoplasmic domain of Tsk 7L has 30 to 40% amino acid sequence identity with the activin and TGF-β type II receptors and contains a predicted kinase domain (*16*) (Fig. 2A). Subdomains I and II contain the conserved motif that constitutes the adenosine triphosphate (ATP) binding site. Comparison of the VIB and VIII domains, especially residues 322 to 344, with other kinases and in particular the transmembrane serine-threonine kinases indicates that Tsk 7L may have serine-threonine kinase activity (*16*). However, the dual specificity kinase activity in vitro of the activin receptor (*17*) suggests that other members of this receptor family may exert not only serine-threonine but also tyrosine kinase activity. Residues 472 to 479 contain a consensus tyrosine kinase target site (*18*), thus Tsk 7L could be a substrate for COOH-terminal tyrosine phosphorylation. There are several potential phosphorylation sites for casein kinase II and protein kinase C. A comparison with all other kinase sequences shows that the Tsk 7L kinase domain is most closely related to the activin and TGF-β type II receptors, indicating that it is a new member of this family of receptor kinases.

The expression of tsk 7L mRNA in various cell lines and tissues was evaluated by Northern blot analysis and showed a widespread distribution. A single 3.5-kb tsk 7L mRNA was present in most cell lines (*19*) and tissues (Fig. 3). The tsk 7L mRNA expression was high in brain, lung, heart, and skeletal muscle, but was not detectable in thymus.

To evaluate the properties of the Tsk 7L receptor protein, we generated expression vectors that can be used for expression studies in mammalian cells and as template for in vitro transcription into tsk 7L mRNA (*20*). Coupled in vitro transcription and translation in reticulocyte lysate in the presence of microsomes resulted in significant amounts of translation product of about 53 kD (Fig. 4), only when the 5′ untranslated region was totally removed (*13*). This size was consistent not only with the deduced amino acid sequence (Fig. 2), but also with the size of the type I receptor, calculated from the electrophoretic mobility of the ^{125}I-labeled TGF-β cross-linked product (*13, 21*).

We evaluated the ability of Tsk 7L to bind TGF-β after transfection of an expression plasmid into monkey COS-1, human 293, and quail QT-6 cells, which all have a high transfection efficiency. Cross-linking of ^{125}I-labeled TGF-β1 to the transfected cells showed little or no increase in TGF-β1 binding, suggesting that Tsk 7L by itself could not bind TGF-β1 (Fig. 5, A and B). Similar cross-linking experiments using ^{125}I-labeled TGF-β2, activin, or inhibin did not show increased ligand binding (*13*).

Overexpression of Tsk 7L unexpectedly

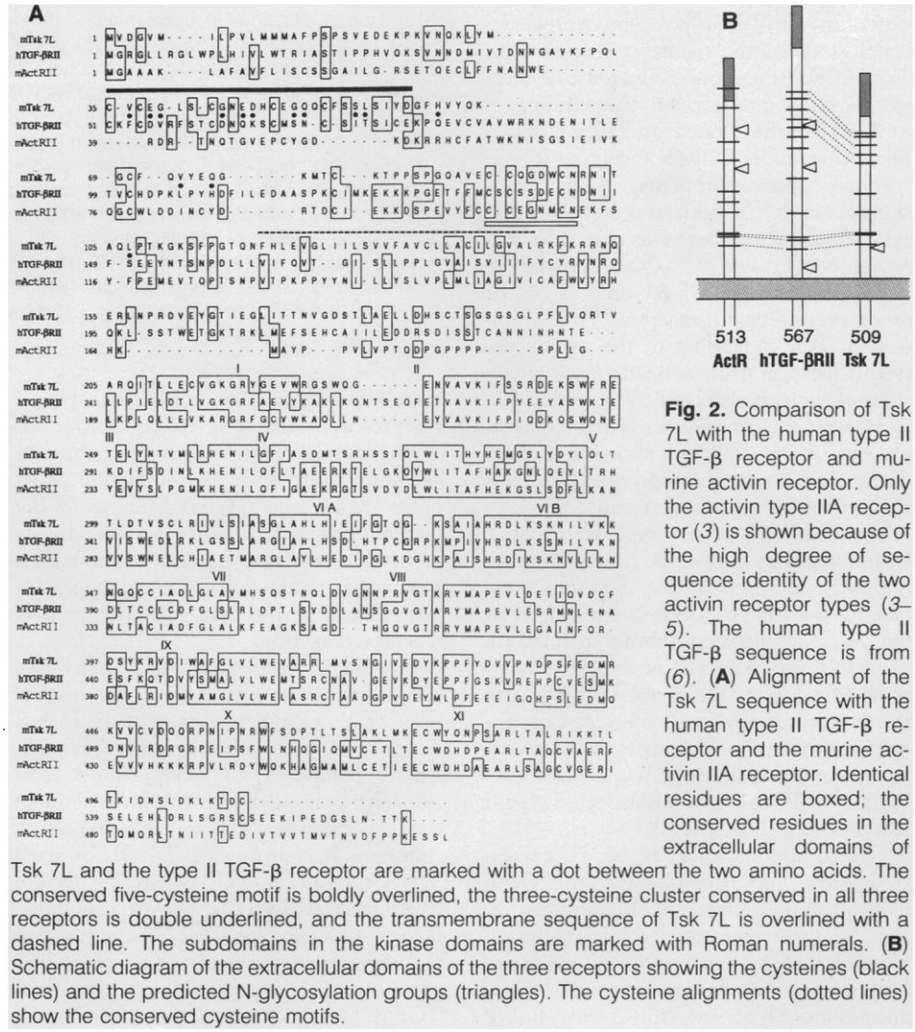

Fig. 2. Comparison of Tsk 7L with the human type II TGF-β receptor and murine activin receptor. Only the activin type IIA receptor (*3*) is shown because of the high degree of sequence identity of the two activin receptor types (*3–5*). The human type II TGF-β sequence is from (*6*). (**A**) Alignment of the Tsk 7L sequence with the human type II TGF-β receptor and the murine activin IIA receptor. Identical residues are boxed; the conserved residues in the extracellular domains of Tsk 7L and the type II TGF-β receptor are marked with a dot between the two amino acids. The conserved five-cysteine motif is boldly overlined, the three-cysteine cluster conserved in all three receptors is double underlined, and the transmembrane sequence of Tsk 7L is overlined with a dashed line. The subdomains in the kinase domains are marked with Roman numerals. (**B**) Schematic diagram of the extracellular domains of the three receptors showing the cysteines (black lines) and the predicted N-glycosylation groups (triangles). The cysteine alignments (dotted lines) show the conserved cysteine motifs.

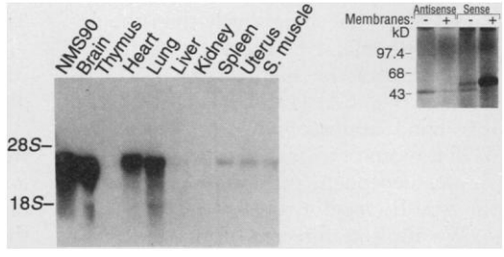

Fig. 3. (**Left**) Northern hybridizations of tsk 7L mRNA in various adult mouse tissues. The positions of the 28*S* and 18*S* ribosomal RNAs are marked. Polyadenylated mRNA (10 μg per lane) was separated in 1.1% agarose, 5% formaldehyde gels. The blot was stripped and reprobed with the human glyceraldehyde phosphate dehydrogenase cDNA and showed equal loading (*19*). **Fig. 4.** (**Right**) Proteins obtained after in vitro transcription of tsk 7L cDNA and subsequent translation in reticulocyte lysate. Runoff transcripts were synthesized with sp6-polymerase from expression plasmids containing tsk 7L cDNA cloned in the antisense or sense orientation. Equal amounts (2.5 μg) of RNA were translated in vitro in a rabbit reticulocyte lysate in the presence of [^{35}S]Met, in the presence (+) or absence (−) of canine microsomal membranes (Promega). The reaction products were analyzed by denaturing gel electrophoresis and autoradiography. The tsk 7L translation product is visible as a 50- to 55-kD band (arrow). The tsk 7L expression plasmids were constructed as described (*27*).

interfered with the binding of TGF-β1 to type II receptors at the cell surface. This was apparent in QT-6 cells, which have heterogeneous type II receptors. Transfection of tsk 7L inhibited binding of TGF-β to the lower type II receptor components (Fig. 5B), but increased its binding to a larger receptor component (Fig. 5B, lane 4). Whereas this result suggests an interference of some sort, the lack of characterization of the quail TGF-β receptors precludes conclusions on the nature of this effect of Tsk 7L. We therefore evaluated the effect of Tsk 7L expression on the binding of TGF-β1 to the cloned and characterized human type II TGF-β receptor (6) in cotransfection experiments, using expression plasmids for both receptors, in 293 cells which lack endogenous type II TGF-β receptors (*11*) (Fig. 5C). Overexpression of Tsk 7L abolished TGF-β1 binding to the overexpressed type II receptor. This inhibition of TGF-β binding to the transfected type II receptor decreased with lower quantities of the cotransfected tsk 7L plasmid in a dose-dependent manner (*13*). Overexpression of Tsk 7L did not affect the amount of transfected type II receptor mRNA (*19*), thus ruling out an effect on transcription or mRNA stability of the type II receptor. Cotransfections of the type II receptor plasmid with pRK5 (*20*), the parent plasmid lacking the tsk 7L cDNA insert (Fig. 5C, lane 6), or an identical plasmid with the tsk 7L cDNA insert in the antisense orientation (*13*), did not affect TGF-β1 binding to the overexpressed type II receptor. Cotransfection of a similar activin expression vector had only a small inhibitory effect on the binding of TGF-β to the transfected type II receptor (Fig. 5C, lane 5).

The inhibitory effect of tsk 7L expression on the binding of TGF-β to the type II receptor was apparent at low concentrations of the transfected tsk 7L expression plasmid. At higher concentrations, this inhibition was accompanied by the appearance of an ^{125}I-labeled TGF-β–cross-linked band of a size similar to that of the type I TGF-β receptor (Fig. 6A). This band migrated somewhat more slowly than the endogenous human type I receptor in the 293 cells, as does the murine type I TGF-β receptor (Fig. 6A) (*13*). The appearance of this band corresponding to the type I TGF-β receptor in cells transfected with tsk 7L was dependent on cotransfection with the type II receptor.

We also cotransfected the tsk 7L expression vector with an expression plasmid encoding the murine type II receptor (*21*), thus combining two isogenic receptors. This resulted in an inhibition of TGF-β binding to the type II receptor and in the appearance of the TGF-β binding type I receptor (Fig. 6B). The ^{125}I-labeled band corresponding to the cross-linked type I receptor appeared at lower concentrations of the tsk 7L expression vector than in cotransfections with the human type II receptor. In fact, TGF-β binding to the recombinant type I receptor was already detectable at concentrations of the tsk 7L plasmid that caused only a moderate inhibition of TGF-β binding to the type II receptor (Fig. 6B).

To further verify that Tsk 7L itself can bind TGF-β, we cotransfected the murine (Fig. 6C) or human (*13*) type II TGF-β receptor with a truncated form of Tsk 7L, which comprises the extracellular and transmembrane domains but lacks most of the cytoplasmic domain. As expected, transfection of truncated Tsk 7L by itself did not result in TGF-β binding to the truncated receptor (*13*). However, cotransfection with the type II receptor resulted in the appearance of a smaller TGF-β binding protein that corresponded to the size of the TGF-β bound truncated form of Tsk 7L (Fig. 6C). The truncated form of Tsk 7L may have a diminished ability to inhibit TGF-β binding to the type II receptor, suggesting that this inhibitory activity may be associated in part with the cytoplasmic domain.

Our data suggest that Tsk 7L and its truncated form require the type II receptor for transport to the cell surface, TGF-β binding or both. In principle, it is possible that expression of Tsk 7L upregulates the endogenous type I receptor and is itself not a TGF-β receptor. However, this is very unlikely because tsk 7L was derived from a murine cell line and the newly expressed TGF-β receptor in 293 cells is slightly larger than the endogenous human type I receptor, as is the mouse type I receptor (Fig. 6B). Furthermore, cotransfection of truncated tsk 7L resulted in the appearance of a much smaller TGF-β binding protein at the cell surface, with a size consistent with the truncated tsk 7L receptor and does not increase the endogenous type I TGF-β receptor level (Fig. 6C).

Our conclusion that tsk 7L corresponds to a type I receptor is based on several observations. The identity of the type I TGF-β receptor has so far only been based on the ability to bind TGF-β and on the 60- to 70-kD size of its ^{125}I–TGF-β–cross-linked band on denaturing polyacrylamide gels (*22*). In transfected cells, Tsk 7L binds TGF-β and the size of the cross-linked receptor encoded by the tsk 7L cDNA is 68 kD (Fig. 6, A and B). The type I receptor itself has been proposed to have a size of about 53 kD (*22*), which is consistent with the size of Tsk 7L obtained by in vitro transcription and translation assay (Fig. 4). The cross-linked type I receptor always migrates as a less diffuse band than the type II receptor, suggesting a lower degree of glycosylation, and this is also the case for the tsk 7L–encoded TGF-β receptor (Fig. 6, A and B). Type I receptors are expressed by almost all cells in culture. This is consistent with the widespread expression of tsk 7L mRNA by cell lines and in adult mouse tissues (*19*) (Fig. 3). Finally, analysis of Mv1Lu mutant cell lines revealed that loss of type II receptors was always accompanied by a lack of type I receptors, suggesting that expression of the type I receptor at the cell surface depends on type II receptor expression (*23*). Furthermore, transfection of the type II receptor in a cell line lacking TGF-β receptors resulted in the appearance of not only type II receptors, but also type I receptors, suggesting that the type I recep-

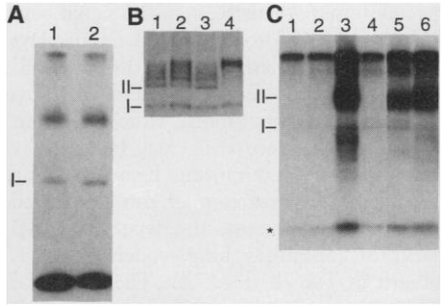

Fig. 5. Binding of ^{125}I-labeled TGF-β and cross-linking to cells transfected with a tsk 7L expression plasmid. (**A**) QT-6 cells were transfected with salmon sperm DNA (lane 1) or the tsk 7L expression vector (*27*) (lane 2). No substantial increase in binding of ^{125}I-labeled TGF-β to the type I receptor (marked I) is apparent. (**B**) QT-6 cells were transfected with salmon sperm DNA (lane 1), a low-level tsk 7L expression plasmid (*13*) (lane 2), an expression plasmid with the tsk 7L cDNA in the antisense orientation (lane 3), and the tsk 7L expression plasmid (*27*) (lane 4). All transfections were done with 20 μg of DNA. I and II, the type I and presumed type II receptors. (**C**) Two hundred ninety-three cells were mock-transfected (lane 1) or transfected with salmon sperm DNA (lane 2). The cells in lanes 3 to 6 were transfected with the human type II receptor expression plasmid (*11*) (20 μg) alone (lane 3) or in the presence of the tsk 7L expression plasmid (20 μg) (lane 4), the murine activin IIA receptor expression plasmid (lane 5) or the parent pRK5 plasmid (*20*) lacking a cDNA insert (lane 6). I and II, type I and II receptors. In these cells, overexpression of the type II receptor frequently results in increased type I receptor levels (*11*). The asterisk marks free ^{125}I-labeled TGF-β. Transfections were done by the calcium phosphate method (*28*). Seventy-two hours after transfection, the cells were incubated with ^{125}I-labeled human TGF-β1, affinity–cross-linked, and the cell lysates were analyzed by denaturing gel electrophoresis and autoradiography. Cross-linking reactions with ^{125}I-labeled TGF-β1 in (A) were done as described (*29*); cross-linking in (B) and (C) was done as described (*30*).

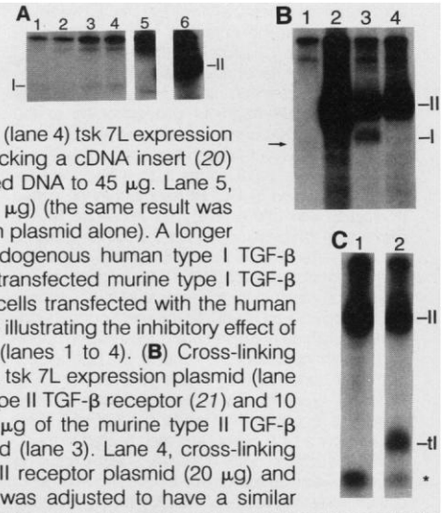

Fig. 6. Binding of ^{125}I-labeled TGF-β and cross-linking to 293 cells cotransfected with the type II receptor. (**A**) The 293 cells were transfected with the human type II TGF-β receptor expression plasmid (*11*) (10 μg) in the presence of 5 μg (lane 1), 15 μg (lane 2), 25 μg (lane 3), or 35 μg (lane 4) tsk 7L expression plasmid. pRK5, the parent expression plasmid lacking a cDNA insert (*20*) was added to bring the total amount of transfected DNA to 45 μg. Lane 5, 293 cells transfected with salmon sperm DNA (30 μg) (the same result was obtained by transfection with the tsk 7L expression plasmid alone). A longer exposure is shown to allow detection of the endogenous human type I TGF-β receptor (arrow), which migrates faster than the transfected murine type I TGF-β receptor (marked I) in lanes 1 to 4. Lane 6, 293 cells transfected with the human type II TGF-β receptor plasmid (20 μg) alone, thus illustrating the inhibitory effect of tsk 7L on TGF-β binding to the type II receptor (lanes 1 to 4). (**B**) Cross-linking patterns of 293 cells transfected with 20 μg of the tsk 7L expression plasmid (lane 1), 20 μg of an expression vector for the murine type II TGF-β receptor (*21*) and 10 μg of the control pRK5 plasmid (lane 2), or 20 μg of the murine type II TGF-β receptor plasmid and 10 μg of the tsk 7L plasmid (lane 3). Lane 4, cross-linking pattern of cells transfected with the murine type II receptor plasmid (20 μg) and control plasmid pRK5 (10 μg), but the loading was adjusted to have a similar intensity of the type II receptor band as in lane 3. Arrow, the endogenous human type I receptor in lane 1; I and II, transfected murine type I and II receptors. (**C**) Binding of ^{125}I-labeled TGF-β1 to the truncated Tsk 7L receptor that was cotransfected with the murine type II receptor. The murine type II receptor expression plasmid (20 μg) was transfected in the absence (lane 1) or presence (lane 2) of the truncated tsk 7L expression plasmid (10 μg). Transfections (*28*) and cross-linking of ^{125}I-labeled TGF-β (*30*) were done as described. II, type II receptor; tI, the ^{125}I-labeled–TGF-β cross-linked truncated type I receptor; asterisk, free ^{125}I–TGF-β1. The endogenous type I receptor is not visible due to the short exposure time.

tor requires the type II receptor for transport to the cell surface, TGF-β binding, or both (*10*). This property of the type I receptor corresponds with our observations that tsk 7L requires cotransfection with the type II receptor to be expressed as a TGF-β receptor at the cell surface. Thus, on the basis of these combined findings, we conclude that tsk 7L encodes a type I TGF-β receptor. This does not preclude the possible existence of additional type I receptors, especially since there may be more than one type II receptor (*21*, *24*).

The ability of the type II and type I receptors to bind the same ligand is likely to depend on similar structural features in the extracellular domains. The five cysteines containing conserved segment close to the NH_2-terminus of both receptors are not present in other serine-threonine kinase receptors and may provide a three-dimensional structure required for TGF-β binding. The faster migration through polyacrylamide gels of the cross-linked type I receptor as a less diffuse band than the type II receptor is presumably due to the absence of defined sequences of the type II receptor and the lower degree of N-glycosylation (Fig. 2, A and B).

The function of Tsk 7L as a type I TGF-β receptor was also associated with an inhibition of TGF-β binding to the type II receptor. This inhibition was apparent with both the human and mouse type II receptors, but seemed more potent with the human type II receptor. In the latter case, less Tsk 7L expression was required for the inhibitory effect than for the appearance of the TGF-β binding type I receptor. Less tsk 7L plasmid was required for detection of type I receptors when mouse, as opposed to human, type II receptors were present. Heterodimerization of both receptors could perhaps decrease the cross-linking efficiency of TGF-β to the type II receptor, but this would not explain all results. Consistent with its dominant negative nature (*25*), this inhibition may result from a type of interaction of Tsk 7L with the type II receptor that results in interference with cell surface transport of the receptor complex, its availability for TGF-β binding, or both. The quantitative differences between the results with the human and mouse type II receptors suggest different affinities of mouse Tsk 7L for the mouse and human type II receptors. A simple model of heterodimerization between the type I and type II receptor has been proposed (*10*), but this model may now have to be expanded. The dual action of Tsk 7L may be explained by invoking the possibility that the complex consists of more components than the two receptor monomers; that more than one type of complex exists; or that cooperative interactions, steric alterations, and resulting affinity changes between the receptors and ligand can occur. The inhibitory effect of Tsk 7L on TGF-β binding to the type II receptor was detected in experiments in which both receptors are overexpressed. Whether Tsk 7L functions as a natural dominant negative inhibitor in cells under normal physiological conditions remains to be verified.

Even though the mechanism for these interactions is unclear, stoichiometric variations in the expression of the type II receptor and Tsk 7L, and other possibly associated proteins, may determine the TGF-β binding to the type I and II receptors. A simple model whereby the type I receptor requires heterodimerization with the type II receptor for TGF-β binding (*10*) would always result in equal or less availability of type I than type II receptors at the cell surface. However, a possible dual function of the type I receptor as TGF-β binding receptor and dominant negative inhibitor allows for wide variations in the ratios of type I and type II receptor availability for TGF-β binding, as has been observed in many cell lines. Because the type I and II receptors are associated with two distinct signaling pathways responsible for the diverse TGF-β activities (*11*), the availability of these two receptors as a result of their interactions could determine the cellular response to TGF-β.

REFERENCES AND NOTES

1. A. Ullrich and J. Schlessinger, *Cell* **61**, 243 (1990).
2. J. D. Scott and T. R. Soderling, *Curr. Opin. Neurobiol.* **2**, 289 (1992).
3. L. S. Mathews and W. W. Vale, *Cell* **65**, 973 (1991).
4. L. Attisano *et al.*, *ibid.* **68**, 97 (1992).
5. L. S. Mathews, W. W. Vale, C. R. Kintner, *Science* **255**, 1702 (1992).
6. H. Lin *et al.*, *Cell* **68**, 775 (1992). Erratum, *ibid.* **70**, following 1068 (1992).
7. A. Roberts and M. B. Sporn, in *Peptide Growth Factors and Their Receptors*, M. B. Sporn and A. B. Roberts, Eds. (Springer-Verlag, Heidelberg, 1990), pp. 419–472.
8. J. Massagué, *Cell* **69**, 1067 (1992).
9. M. Laiho, F. M. B. Weiss, J. Massagué, *J. Biol. Chem.* **265**, 18518 (1990).
10. J.-F. Wrana *et al.*, *Cell* **71**, 1003 (1992).
11. R.-H. Chen, R. Ebner, R. Derynck, *Science* **260**, 1335 (1993).
12. The NMS 90 cells were derived from murine NMuMG epithelial cells, which express predominantly type I receptors (*31*). The NMuMG cells were grown to approximately passage 200 in high glucose Dulbecco's minimum essential medium (DMEM), fetal calf serum (7.5%), bovine insulin (10 μg/ml), and 1× nonessential amino acids (Gibco). These cells were then adapted to suspension by dispersion and growth in spinner flasks in high glucose DMEM, equine serum (7.5%), bovine insulin (10 μg/ml), 20 mM Hepes (pH 7.2), and 0.05 to 0.1% Pluronic F-68. Over the course of a year, several cell cultures adapted, after a crisis period, to growth in suspension. Initially, the cells grew as clumps, which varied in sizes depending on the culture isolate. Gradually several isolates started to grow as single cell suspensions. NMS-90 is one of the isolates that had a fast growth rate, grew as single cells in suspension, and expressed many type I TGF-β receptors.
13. R. Ebner and R. Derynck, unpublished data.
14. N. J. Proudfoot, *Trends Biochem. Sci.* **14**, 105 (1989).
15. G. von Heijne, *Nucleic Acids Res.* **14**, 4683 (1986).
16. S. K. Hanks, A. M. Quinn, T. Hunter, *Science* **241**, 42 (1988).
17. T. Nakamura *et al.*, *J. Biol. Chem.* **267** (26), 18924 (1992).
18. R. A. Lindberg, A. M. Quinn, T. Hunter, *Trends Biochem. Sci.* **17**, 114 (1992), A. Bairach, *PROSITE: A Dictionary of Protein Sites and Patterns*, (University of Geneva, Release 7.10, 1991).
19. A. R. Lopez, R. Ebner, R. Derynck, unpublished data.

20. J. L. Graycar *et al.*, *Mol. Endocrinol.* **3**, 1977 (1989)
21. S. Lawler *et al.*, in preparation.
22. F. T. Boyd *et al.*, *J. Cell Sci. Suppl.* **13**, 131 (1990)
23. F. T. Boyd and J. Massagué, *J. Biol. Chem.* **264**, 2272 (1989).
24. S. Cheifetz *et al.*, *ibid.* **265**, 20533 (1990).
25. I. Herskowitz, *Nature* **329**, 219 (1987).
26. Complementary DNAs corresponding to nucleotides 887 to 1244 and 671 to 1436 of the activin receptor IIA (*3*) were obtained by reverse transcriptase coupled to the PCR (*32*) with rat GH3 cell RNA as substrate These cDNA fragments that encode the cytoplasmic domain sequences conserved between the activin receptors and the *Caenorhabditis elegans* Daf-1 protein (*33*) were ^{32}P-labeled and used as hybridization probes under low stringency conditions (*34*) to screen λgt10-based cDNA libraries derived from NMS 90 and NIH 3T3 cell RNA. Complementary DNA inserts of hybridizing clones were subcloned into M13 mp19 and subjected to dideoxy sequencing (*35*). The incidence of tsk 7L cDNAs in the NMS 90 cDNA library was 3 per 1000 recombinant phage. Two full-size tsk 7L cDNAs, one from each cDNA library, were sequenced in their entirety with the Sequenase reagents (United States Biochemical) or, for the GC-rich 5′ sequence, Taq-polymerase, gene 32 single strand binding protein (Pharmacia), and 7-deaza-dGTP (Boehringer Mannheim).
27. The tsk 7L expression plasmid was generated as follows. A cDNA fragment corresponding to the full-size coding sequence and the partial 3′ untranslated sequence, yet lacking the entire 5′ untranslated sequence (nucleotides 1 to 496) was subcloned as a Hind III–Eco RI fragment in the sense orientation into pRK7 (*20*). The antisense tsk 7L expression plasmid contained the full-size tsk 7L cDNA inserted as an Eco RI fragment in the antisense orientation into pRK5 (*20*). To generate an expression plasmid for the truncated tsk 7L receptor, an Eco RI–Eco RV cDNA fragment corresponding to nucleotide −10, preceding the initiation codon of tsk 7L, to nucleotide 464, that is, the last nucleotide of the codon for Glu^{155}, was generated by PCR. A double-stranded oligonucleotide adaptor for the sequence encoding the epitope tag FLAG (*36*) followed by a stop codon was then ligated to the 3′ end of the truncated tsk 7L cDNA, and the resulting fragment was inserted into the Eco RI and Xba I sites of the expression vector pRK5 (*20*), thus generating the expression plasmid for the truncated Tsk 7L receptor. To generate the activin IIA receptor expression plasmid, the 2 6- kbp Xba I–Bam HI cDNA fragment (*3*) was subcloned into pRK7 (*20*).
28. C. Gorman, R. Padmanabhan, B. H. Howard, *Science* **221**, 551 (1983).
29. X.-F. Wang *et al.*, *Cell* **67**, 797 (1991)
30. D. Gazit *et al*, *Mol. Endocrinol.* **7**, 189 (1993).
31. P. R. Segarini *et al.*, *ibid.* **3**, 261 (1989).
32. D. A. Rappolee, D Mark, M. J. Banda, Z. Werb, *Science* **241**, 708 (1988).
33. L. L. Georgi, P. S. Albert, D. L. Riddle, *Cell* **61**, 635 (1990).
34. R. Derynck *et al.*, *EMBO J.* **7**, 3737 (1988).
35 A. J. H. Smith, *Methods Enzymol.* **65**, 560 (1980).
36. T P. Hopp *et al.*, *Biotechnology* **6**, 1204 (1988).
37. We thank R. Weinberg and W Vale for the human type II TGF-β receptor and murine activin receptor IIA cDNAs; S. Reeves, L. Moser, and V. Sung for their contributions; C. Meneses for technical support; and K. Yamamoto and Z. Werb for helpful discussions. Supported by the Deutsche Forschungsgemeinschaft (R.E.), the Tobacco-Related Disease Research Program of California (L.S.), a Veterans Administration Career Development Award (A.R L.), and grants from the ACS and NIH to R D

11 March 1993; accepted 22 April 1993