Distinct Roles of the Intracellular Domains of Transforming Growth Factor-β Type I and Type II Receptors in Signal Transduction*

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Transforming growth factor-β (TGF-β) transduces signals through binding to type I (TβR-I) and type II (TβR-II) serine/threonine kinase receptors. TβR-I requires TβR-II for ligand binding, whereas TβR-II requires TβR-I for signaling. We generated two different chimeric TGF-β receptors, i.e. TβR-1.2 containing the extracellular domain of TβR-I and the intracellular domain of TβR-II, and TβR-2.1 containing the extracellular domain of TβR-II and the intracellular domain of TβR-I. TβR-2.1 bound 125I-TGF-β1 alone, whereas TβR-1.2 bound the ligand only in the presence of TβR-II or TβR-2.1. When transfected into a mutant mink lung epithelial cell line that lacks functional TβR-II, TβR-II cDNA, but not TβR-2.1 cDNA, restored the responsiveness to TGF-β1 with regard to transcriptional activation of plasminogen activator inhibitor-1 gene promoter and 12-O-tetradecanoylphorbol-13-acetate-responsive elements. In a mutant mink lung epithelial cell line lacking TβR-I, TβR-I cDNA stimulated promoter activity, but the TβR-1.2 cDNA did not. TβR-2.1 formed an oligomer with TβR-II when transfected into COS cells, but the complex did not transduce the signal after ligand stimulation. On the other hand, co-transfection of TβR-1.2 and TβR-2.1 cDNAs restored the responsiveness to TGF-β1. These results indicate that an interaction between the intracellular regions of TβR-I and TβR-II, triggered by ligand binding to the extracellular domains of these receptors, leads to efficient signal transduction by TGF-β.

Transforming growth factor-β (TGF-β) is a family of three 25-kDa proteins (TGF-β1, -β2, and -β3) that regulate the growth, differentiation, migration, and adhesion of various cell types (1). TGF-βs are structurally related to a number of other factors that also regulate the growth and differentiation of different cell types, including activins and inhibins, bone morphogenetic proteins, Mullerian inhibiting substance, and glial cell line-derived neurotrophic factor (2, 3). TGF-βs exert their effects through binding to various cell surface receptors and binding proteins (2, 3). Among these, type I (TβR-I) and type II (TβR-II) receptors are most important for signal transduction. After ligand binding, TβR-I and TβR-II form a heteromeric receptor complex, and transduce signals (4–7). Thus, TβR-II can bind ligands in the absence of TβR-I but requires TβR-I for signaling. TβR-I, on the other hand, cannot bind ligands in the absence of TβR-II. Recent data suggest that ligand binding induces formation of a hetero-oligomeric receptor complex, most likely a heterotetramer composed of two molecules each of TβR-I and TβR-II (8). Moreover, it was shown that TβR-II is present as a homo-oligomer, presumably a homodimer, in the absence of the ligand (9, 10).

Both TβR-I and TβR-II are serine/threonine kinase receptors (11–14). The overall structures of TβR-I and TβR-II are similar and consist of relatively short extracellular domains with cysteine-rich regions, followed by single transmembrane domains, and intracellular regions containing serine/threonine kinase domains. The serine/threonine kinase domains of TβR-I and TβR-II have 41% amino acid sequence similarity, but the sequence similarities are less in other parts of the cytoplasmic domains. Two kinase inserts are observed at analogous positions in the kinase domains of TβR-I and TβR-II. In the region preceding the kinase domain of TβR-I, there is a glycine- and serine-rich sequence, termed GS domain, which is conserved in other type I receptors for proteins in the TGF-β superfamily (2, 3), but is not present in TβR-II. After the C termini of the kinase domains, TβR-II has a tail of 24 amino acid residues, whereas that of TβR-I is composed of only 5 amino acid residues.

The functional roles of the intracellular regions of TβR-I and TβR-II are not fully elucidated. The kinase activities of type II and type I receptors are essential for signaling activity at least for the growth inhibition signal (6, 13, 15, 16). However, the C-terminal tail and the kinase inserts of TβR-II are not required for signal transduction (17). The serine/threonine kinase of TβR-II is constitutively active. Recent data suggest that after ligand binding and formation of a heteromeric receptor complex, TβR-II transphosphorylates the GS domain of TβR-I, which may then activate the TβR-I kinase (15).

In order to investigate whether the intracellular domains of TβR-I and TβR-II have distinct functional roles in the heterooligomeric complex, or whether they are interchangeable, we generated two different chimeric receptors in which the intracellular domains of TβR-I and TβR-II, triggered by ligand binding to the extracellular domains of these receptors, leads to efficient signal transduction by TGF-β.

EXPERIMENTAL PROCEDURES

Construction of TGF-β Receptor Chimeras—cDNAs for human TβR-II (11) and TβR-I (12) were used to create the chimeric TGF-β receptor constructs, TβR-1.2 and TβR-2.1. A polymerase chain reaction (PCR) product encompassing the extracellular and transmembrane domains of TβR-I (amino acids 1–153), flanked by an EcoRI site in the N terminus and an HpaI site in the C terminus, was obtained using a Perkin-Elmer DNA Thermal Cycler with Pyrococcus furiosus DNA polymerase (Stratagene) and with linearized human TβR-I cDNA as a template. The primers encompassed nucleotides 299–3205 of the TβR-I cDNA. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: TGF-β, transforming growth factor-β; TβR, TGF-β receptor; PCR, polymerase chain reaction; GS domain, Gly/Ser-rich domain.
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FIG. 1. Binding of 125I-TGF-β1 to wild-type and chimeric TGF-β receptors. Wild-type and chimeric TGF-β receptors were transfected into COS cells, and the binding of 125I-TGF-β1 was analyzed by affinity labeling and cross-linking. Samples were analyzed by SDS-gel electrophoresis and autoradiography directly (IP (−); lanes 1–8) or after immunoprecipitation using antisera to TpR-I or TpR-II (IP (+); lanes 10–18). The antisem to TpR-I was used in lanes 10, 11, 14, 15, 17, and 18; the TpR-II antisem was used in lanes 12, 13, and 16. R-I, TpR-I; R-II, TpR-II; R-1.2, TpR-1.2; R-2.1, TpR-2.1. Migration distances of markers of molecular mass are shown to the left.

RESULTS

Ligand Binding Properties of Chimeric TGF-β Receptors—In order to investigate the ligand binding properties of the chimeric receptors TpR-I.2, which has the TpR-I extracellular domain and the TpR-II intracellular domain, and TpR-2.1, which has the TpR-II extracellular domain and the TpR-I intracellular domain, cDNAs for chimeric and wild-type receptors were transfected into COS-1 cells, and the binding of 125I-TGF-β1 was investigated by affinity cross-linking (Fig. 1, lanes 1–9). TpR-I and TpR-1.2 did not bind the ligand when transfected singly, whereas TpR-II and TpR-2.1 bound TGF-β1. Co-transfection of TpR-II or TpR-2.1 cDNAs with TpR-I or TpR-1.2 cDNAs allowed TpR-I and TpR-1.2 to bind the ligand. After co-transfection of TpR-II and TpR-I cDNAs, cross-linking of complexes was performed using the plasmids containing TGF-β receptor cDNAs. Luciferase activities in the cell lysates after stimulation by various concentrations of TGF-β1 were measured using the luciferase assay system (Promega) and a luminometer (model 1250; LKB) (6).
and cross-linked complexes were first immunoprecipitated by the antiserum to TPR-I, which recognizes TPR-2.1, and then immunoprecipitated by the TPR-I antisera (Fig. 2). The TPR-I cross-linked complex could be observed after the sequential immunoprecipitation (Fig. 2). The TPR-I complex could be gated using the p3TP-Lux transcriptional activation assay. However, the functional roles of the intracellular domains of TPR-I1 and TPR-I, both of which have serine/threonine kinase activity, have not been fully elucidated. The present data show that homo-oligomerization of TPR-I intracellular domains, achieved by the transfection of the TPR-2.1 cDNA into the DR mutant cells containing endogenous wild-type TPR-I, or homo-oligomerization of the TPR-II intracellular domains, achieved by the transfection of TPR-1.2 cDNA into the R mutant cells containing endogenous wild-type TPR-II, do not efficiently transduce intracellular signals (Table 1 and Fig. 4). In contrast, hetero-oligomerization of the intracellular domains of TPR-II and TPR-I, achieved by simultaneous expression of TPR-I and TPR-II, or TPR-1.2 and TPR-2.1, transduces signals. These results suggest that the intracellular domains of both TPR-I and TPR-II have distinct functions and both are required for signal transduction.

We have recently shown that TPR-II and TPR-I form a hetero-oligomer, most likely a heterotetramer, after ligand binding (8). Moreover, TPR-II has been shown to form a homo-oligomer in the presence and absence of the ligand (9, 10). However, the homo-oligomerization of TPR-II observed in the R mutant cells are not enough for signal transduction (9). We show here that TPR-2.1, which can form a hetero-oligomer with TPR-II, did not induce any transcriptional response by TGF-β after transfection into the R mutant (Fig. 3C). Since TPR-II forms a homo-oligomer in the absence of the ligand, TPR-2.1 may form a heteromeric complex with TPR-II without the ligand. We did not observe any significant increase in the luciferase activity without ligand stimulation (data not shown); however, the possibility that certain signals other than the p3TP-Lux signal are transduced by the TPR-II and TPR-2.1 complex in the absence of the ligand has not been ruled out. These results suggest that ligand-induced association of the intracellular domains of TPR-I and TPR-II to higher orders of oligomers may be important for efficient signal transduction.

The mechanism of activation of the kinase domains of the TGF-β receptor complex is not fully determined. The ligand initially binds to TPR-II, which then recruits TPR-I to the ligand-TPR-II complex. Recent data revealed that TPR-II transphosphorylates the GS domain of TPR-I, which then triggers the activation of TPR-I kinase (15). Type I receptors appear to act downstream of the type II receptors and specify the properties of intracellular signals (21). Type I receptors, but not type II receptors, were shown to interact with FKBP-12, a binding protein of the macrolyde FK506 and rapamycin (22); thus, FKBP-12 may act downstream of type I receptors in signal transduction.

The present data show that homo-oligomerization of the intracellular domains of TPR-II and TPR-I are required for signal transduction with regard to the p3TP-Lux transcrip-
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#### Table I

| cDNAs transfected<sup>a</sup> | TGF-β receptors expressed | Intracellular domains expressed | Signal in response to TGF-β1 |
|-------------------------------|--------------------------|-------------------------------|-----------------------------|
| Transfection into R mutant cells | R-I | R-I + R-II | R-I | ++ |
| R-1.2 | R-I + R-2.1 | R-I | - |
| R-2.1 | R-I + R-1.2 | R-I + R-II | - |
| Transfection into DR mutant cells | R-I | R-II + R-I | R-I + R-II | ++ |
| R-1.2 | R-II + R-1.2 | R-II | - |
| R-2.1 | R-II + R-2.1 | R-I + R-II | - |
| R-1.2 + R-I | R-II + R-I + R-2.1 | R-I + R-II | - |
| R-1.2 + R-2.1 | R-I + R-2.1 + R-2.1 | R-I + R-II | + |

<sup>a</sup> Since neither of the receptors contain the extracellular domain of TβRII, TGF-β1 does not bind these receptors.

<sup>b</sup> Interaction of R-I and R-2.1 may occur through a ligand-independent association of the TβRII extracellular domain (9, 10).

![Diagram](https://example.com/diagram.png)

**Fig. 4.** Schematic illustration of the signal transduction by chimeric TGF-β receptors. Binding of TGF-β to the TβRI-I and TβRII extracellular domains induces the formation of a hetero-oligomeric receptor complex, presumably a heterotetramer (8), as shown in the figure. For ligand binding to TβRI-I or TβRI-I2, TβRII or TβRI-2.1 are required. The combination of TβRI-I and TβRII, as well as that of TβRI-1.2 and TβRI-2.1, transduce a TGF-β-signal recorded as p3TP-Lux promoter activation. On the other hand, combinations of TβRI-I and TβRI-2.1 or that of TβRI-1.2 and TβRII do not efficiently transduce the signal. I, TβRI-I; II, TβRI-I2; 1.2, TβRI-1.2; 2.1, TβRII-2.1.

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