

Crystal structure of the human T β R2 ectodomain–TGF- β 3 complex

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Transforming growth factor- β (TGF- β) is the prototype of a large family of structurally related cytokines that play key roles in maintaining cellular homeostasis by signaling through two classes of functionally distinct Ser/Thr kinase receptors, designated as type I and type II. TGF- β initiates receptor assembly by binding with high affinity to the type II receptor. Here, we present the 2.15 Å crystal structure of the extracellular ligand-binding domain of the human TGF- β type II receptor (ecT β R2) in complex with human TGF- β 3. ecT β R2 interacts with homodimeric TGF- β 3 by binding identical finger segments at opposite ends of the growth factor. Relative to the canonical 'closed' conformation previously observed in ligand structures across the superfamily, ecT β R2-bound TGF- β 3 shows an altered arrangement of its monomeric subunits, designated the 'open' conformation. The mode of TGF- β 3 binding shown by ecT β R2 is compatible with both ligand conformations. This, in addition to the predicted mode for TGF- β binding to the type I receptor ectodomain (ecT β R1), suggests an assembly mechanism in which ecT β R1 and ecT β R2 bind at adjacent positions on the ligand surface and directly contact each other via protein–protein interactions.

Transforming growth factor β (TGF- β) and other polypeptides of the TGF- β superfamily are ~25 kDa disulfide-linked homodimers that share a common structural scaffold^{1–6} and play critical roles in maintaining cellular homeostasis and controlling cell fate⁷. TGF- β signaling is initiated by binding of the growth factor ligand to the small extracellular domains of two homologous, functionally distinct transmembrane receptor kinases: the TGF- β type I and type II receptors (T β R1 and T β R2, respectively). TGF- β initiates receptor assembly by binding with high affinity to T β R2. T β R1 is subsequently recruited by the T β R2–ligand complex, allowing T β R2 to phosphorylate T β R1. In turn, T β R1 phosphorylates cytoplasmic effectors known as SMADs^{8,9}. Structures of free (unbound) ligands of the TGF- β superfamily^{1–6,10–12}, the type II mouse activin receptor ectodomain (ecActR2)¹³ and the extracellular domain of the BMP receptor 1A (ecBR1A) bound to BMP-2 (ref. 14) have been determined, but no structural information about a type II receptor–ligand complex has been available until now.

Receptor and ligand folds

The X-ray structure of the complex was determined by MIRAS phasing methods and refined to an R-factor of 20.1% ($R_{\text{free}} = 21.5\%$; Table 1). The ecT β R2 fold is compact, consisting of nine β -strands and a single α -helix stabilized by a network of six intrastrand disulfide bonds (Fig. 1a,b). The folding topology

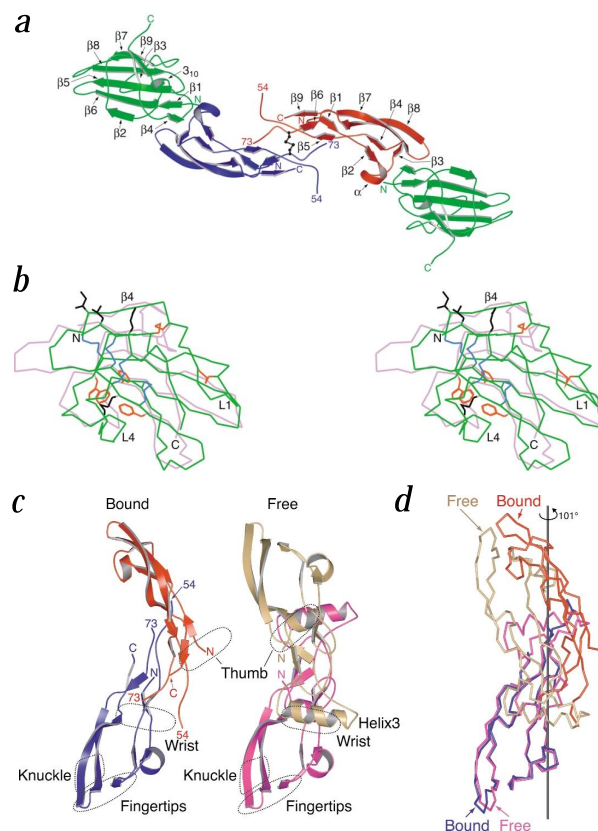


Fig. 1 Structure of the (ecT β R2)₂–TGF- β 3 complex. **a**, Side view of the complex, with two ecT β R2 molecules (green) and the two TGF- β 3 monomers (blue and red). The TGF- β 3 interchain disulfide bond is shown in black. The major secondary structural elements, as determined by the DSSP algorithm⁴², and chain termini are labeled. **b**, Stereo view of ecT β R2 (green) superimposed with ecActR2 (pink). The four ecT β R2 disulfide bonds structurally equivalent to those in ecActR2 — 28–61, 54–78, 98–113 and 115–120 — are shown in blue. The two disulfides present within ecT β R2 loop 1 (L1), 31–48 and 38–44, but not in ecActR2 are shown in red. The one disulfide present within ecActR2, 66–85, but not ecT β R2 is shown in black. Side chains of the three ecT β R2 residues at the interface with TGF- β 3, Leu 27, Ile 53 and Glu 119, are shown in black. β 4 designates β -strand 4 that binds in the cleft between the TGF- β 3 fingertips. ecActR2 residues that comprise the putative interface with activin, Phe 42, Trp 60 and Phe 83, are shown in red. ecT β R2 loop 4 (L4) packs against the surface of the central β -sheet, decreasing the accessibility of ecT β R2 residues structurally equivalent to ecActR2 Phe 42, Trp 60 and Phe 83. **c**, Structural differences between bound and free TGF- β 3. ecT β R2-bound (blue and red monomers) and free (magenta and tan monomers) TGF- β 3 homodimer structures are depicted by the left and right images, respectively. Shown within a single monomer are the 'fingertips' and the BMP type IA and putative type II receptor binding epitopes, designated as the 'wrist' and 'knuckle', respectively. For clarity, the 'thumb' epitope is shown on the symmetry-related monomer. **d**, Comparison of the free and bound TGF- β 3 homodimer structures, in which the structures have been aligned by maximizing the alignment between the blue monomer of the bound form and the magenta monomer of the free form (1.1 Å root mean square (r.m.s.) deviation). Relative to the second monomer of free TGF- β 3 (tan), the second monomer of the ecT β R2-bound form (red) is rotated 101° around the axis shown.

includes a central five-stranded antiparallel β -sheet (26587), eight-residues long at its center, covered by a second layer consisting of two segments of two-stranded antiparallel β -sheets (β 1– β 4, β 3– β 9) (Fig. 1a). Disulfide bonds between Cys residues 28–61, 54–78, 98–113 and 115–120 link this second layer to the central five-stranded β -sheet (Fig. 1b). β -strand 4 forms a sur-

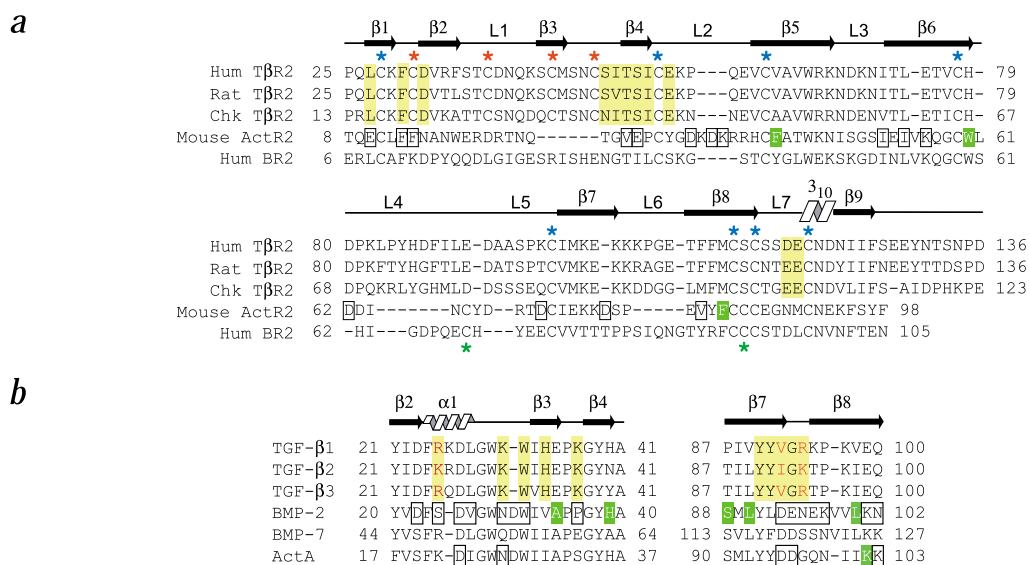


Fig. 2 Sequence alignment of TGF- β superfamily members and type II receptor ectodomains. **a**, Composite amino acid sequence alignment of the extracellular domains of the TGF- β , activin and BMP type II receptors. TβR2 sequences from different species (Hum – *Homo sapiens*, Rat – *Rattus norvegicus* and Chk – *Gallus gallus*) and the human BMP type II receptor (BR2) are aligned on sequence similarity, whereas the mouse activin type II receptor (ActR2) is aligned on the basis of structural homology between ecActR2 (ref. 13) and human ecTβR2. Yellow shading designates interfacial amino acid residues in the (ecTβR2)₂–TGF-β3 complex, green shading designates residues identified by mutagenesis as comprising part of the ActR2 ligand binding surface¹⁵ and the open boxes designate ActR2 residues examined by mutagenesis that do not significantly perturb activin A binding¹⁵. The eight Cys residues designated by blue asterisks form four disulfide bonds that are structurally conserved in ecTβR2 and ecActR2. Red and green asterisks designate Cys residues that form disulfide bonds that are unique to either ecTβR2 or ecActR2, respectively. **b**, Composite amino acid sequence alignment of ligands of the TGF- β superfamily. The three major isoforms of human TGF-β1–4, BMP-2 (ref. 5) and BMP-7 (ref. 6) are aligned on the basis of structural homology, whereas the activin A (ActA) alignment is based on sequence homology. Yellow shading indicates the amino acid residues at the ecTβR2–TGF-β3 interface. Residues at the interface, but variable among the three major isoforms of TGF-β, are indicated in red text. BMP-2 and ActA residues shaded green correspond to those identified through mutagenesis as comprising part of the binding sites for ecBR2 (ref. 21) and ecActR2 (ref. 43), respectively. Open boxes designate BMP-2 and ActA residues examined by mutagenesis that do not significantly perturb ecBR2²¹ or ecActR2 (ref. 43) binding affinities, respectively.

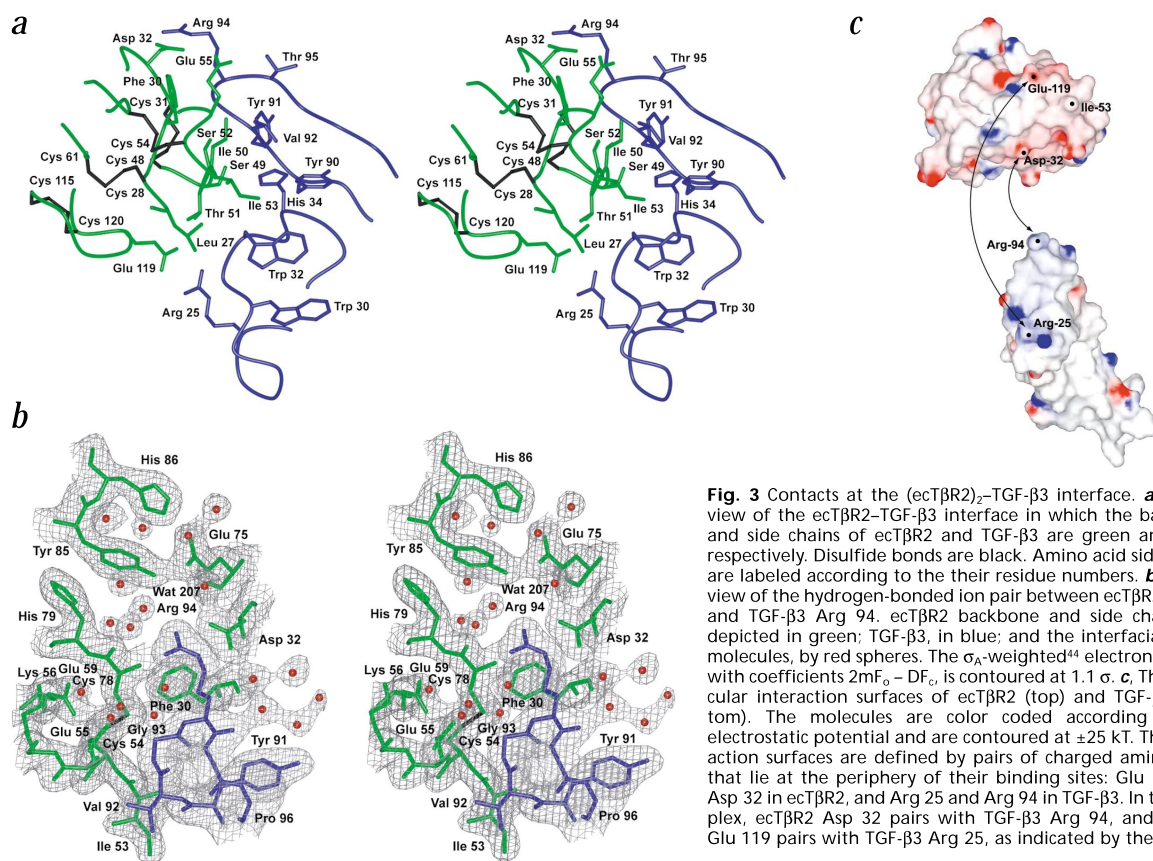
face ridge that binds in a cleft between the ligand fingertips (see below). Absent in the ecTβR2 structure are the N- and C-terminal segments, residues 15–24 and 131–136, respectively, which are disordered in the crystal.

As expected from sequence analysis, the folding topology and the disulfide bonds mentioned above are conserved in ecActR2 (ref. 13) (Fig. 1b). The principal differences in structure include two loop insertions in ecTβR2. The first follows the second β-strand and forms an antiparallel structure separated by ~6 Å and stabilized by two intraloop disulfide bonds, 31–48 and 38–44 (loop 1). This loop replaces a shorter one in ecActR2, which is oriented in the opposite direction and devoid of disulfide crosslinks. The second insertion increases the length of the loop connecting β-strands 6 and 7 (loop 4) by seven residues relative to ecActR2 so that it packs against the solvent-exposed surface of the central β-sheet and diminishes the accessibility of Val 62, His 79 and Met 112. These ecTβR2 residues are structurally equivalent to ecActR2 residues Phe 42, Trp 60 and Phe 83, which comprise the putative activin binding site¹⁵. The alternate modes of ligand recognition by ecActR2 and ecTβR2 seem, therefore, to be a result of sequence divergence of the type II receptors.

ecTβR2-bound TGF-β3 adopts an extended fold in which the monomers comprise one α-helix and nine β-strands stabilized by a cysteine knot motif. The two monomers that form the biologically active homodimer assume a head-to-tail arrangement linked by a disulfide bond at position 77 (Fig. 1a). The monomer fold, described by analogy to that of a curled hand², includes fingers 1–4 formed by β-strands 2, 4, 7 and 8, respectively, and the

palm, formed by β-strands 1, 5, 6 and 9. Comparison of the free⁴ and ecTβR2-bound TGF-β3 monomer structures reveals a slight movement of the β-turn connecting fingers 3 and 4 away from fingers 1 and 2. The overall structure of the palm and finger components of the bound monomer closely resembles that of the free monomer (Fig. 1d).

Other perturbations in the ecTβR2-bound TGF-β3 structure relative to that of free TGF-β3 are more pronounced. Absent in the structure bound form are the thumb, the N-terminal segment including a one-turn helix (helix 1) and the wrist, the central three-turn α-helix (helix 3) positioned between β-strands 5 and 6. These regions, residues 1–12 and 55–72, respectively, are disordered in the crystal and could not be modeled (Fig. 1c). Accompanying the disruption of these structural elements is a rearrangement of the two TGF-β3 monomers. One of the monomers in the bound form undergoes a rotation of 101° around an axis approximately colinear with the direction of its β-strands relative to the other monomer (Fig. 1d). The observed homodimer structural transition is probably coupled to the disruption of α-helix 3, because helix 3 in unbound TGF-β3 contributes 58% (680 Å² per monomer) of the buried surface area and the majority of hydrophobic contacts at the homodimer interface⁴. Circular dichroism (CD)¹⁶ and NMR¹⁷ studies of the free ligand indicate that TGF-β3 consists of a stable disulfide-bonded core region comprising fingers 1–4 and a labile palm/wrist region, which is composed of α-helix 3 and the connecting loop segments at its N- and C-terminal ends. Together, these observations suggest that the observed TGF-β3 conforma-



tional change is related to the intrinsic conformational flexibility of TGF-β3.

The TβR2-TGF-β3 interface

In the complex, a surface ridge formed by residues Ser 49–Cys 54 of TβR2 (β-strand 4) binds in a cleft between the tips of the fingers of the TGF-β3 ligand (Fig. 3a). The cleft is bordered on one side by the single-turn α-helix that links finger 1 with finger 2 and on the other side by the β-turn that links finger 3 and finger 4. Each bound ecTβR2 molecule contacts only amino acids within a single TGF-β3 monomer (Fig. 1a). The receptor-bound form of the ligand retains two-fold symmetry, and the C-termini of the receptors are separated by 92 Å. Buried at the ecTβR2-TGF-β3 interface is 515 Å² of solvent-accessible surface area per polypeptide. Involved in binding are 11 residues from ecTβR2 and nine residues from TGF-β3. Among the interfacial residues in ecTβR2, all are identical in the human, rat and chicken TβR2 sequences, except for conservative replacements that occur at positions 49 and 50 (Fig. 2a). Among the nine interfacial residues in TGF-β3, there are no differences compared to TGF-β1, consistent with the noted similarity in TGF-β1 and TGF-β3 affinities toward binding TβR2 (Fig. 2b). In TGF-β2, three interfacial positions are substituted relative to TGF-β3, accounting for the diminished affinity of TGF-β2 toward binding TβR2 (see below).

ecTβR2 residues Leu 27 and Phe 30 in β-strand 1, and Ile 50, Thr 51 and Ile 53 in β-strand 4 form a convex hydrophobic ridge that inserts into the concave cleft formed by TGF-β3 residues Trp 32 from fingers 1–2, and Tyr 90, Tyr 91 and Val 92 from fin-

gers 3–4 (Fig. 3a). The hydrophobic cavity formed by TGF-β3 residues Trp 32, Tyr 90 and Val 92 is structurally conserved in other ligands of the TGF-β superfamily^{1–6} and is filled only partially by Ile 53 of ecTβR2. In the crystal structure of the uncomplexed TGF-β3, a dioxane molecule from the crystallization mother liquor is observed in this cavity⁴. In the crystal structure of the (ecBR1A)₂-BMP-2 complex, the cavity is partially occupied by Phe 85 from ecBR1A, an invariant residue in type I receptors of the superfamily¹⁴ (see below).

The ligand fingertips each contribute a single positively charged amino acid, Arg 25 and Arg 94, that is involved in the formation of hydrogen-bonded ion pairs with ecTβR2 residues Glu 119 and Asp 32, respectively (Fig. 3a–c). These are the only two pairs of charged residues at the interface; they are located opposite one another at the periphery of the binding site (Fig. 3c). These residues are either variable or poorly conserved among different ligands and type II receptors of the TGF-β superfamily (Fig. 2a,b), suggesting that they play a role in determining binding specificity. Analysis of the TGF-β isoform chimeras constructed to identify residues among the three isoforms responsible for the differences in TβR2 binding affinities establish that Arg 94 is important^{18–20}. Residue 94 is an Arg in TGF-β1 and TGF-β3 but a Lys in TGF-β2. The structure of the complex explains the exquisite selectivity shown by TβR2 toward binding an Arg residue at this position of the ligand. Arg 94 of TGF-β3 and Asp 32 of ecTβR2 are oriented such that two nearly ideal side chain hydrogen bonds, which cannot be supported by any other ion pair, are formed between the Ne and Nη1 atoms of the Arg and the side chain carboxylate of the

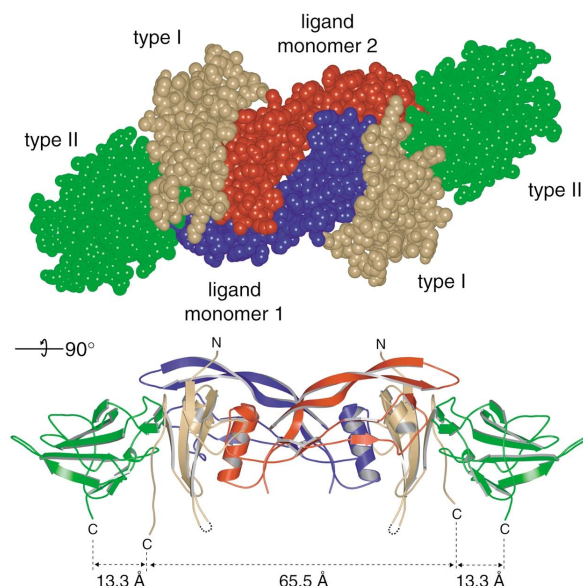


Fig. 4 Model for the relative positioning of the type I and type II receptor ectodomains in the TGF- β -receptor signaling complex. The model is based upon superposition of the ligand components of the ecT β R2-TGF- β 3 and ecBR1A-BMP-2 complexes¹⁴. It was constructed by fixing the coordinates of the ecBR1A-BMP-2 complex and then aligning the contents of the ecT β R2-TGF- β 3 asymmetric unit such that the r.m.s. deviation in backbone atom positions between residues 88–98 in TGF- β 3 and residues 89–100 in BMP-2 were minimized. This operation is repeated using residues 89–100 in the symmetry-related BMP-2 monomer to generate the full (ecT β R2)₂-(ecBR1A)₂-BMP-2 complex. The model is presented as a space filling (top) or as a ribbon diagram rotated by 90° (bottom) in which the two BMP-2 monomers are depicted in red and blue; the two ecBR1A molecules, in tan; and the two ecT β R2 molecules, in green. The chain termini are labeled.

Asp (Fig. 3b). The Asp 32–Arg 94 ion pair is further stabilized by ecT β R2 residues Glu 75 and Tyr 85, which interact with Asp 32 through a tightly bound water molecule (Fig. 3b). This interaction restricts the rotation of Asp 32 such that its side chain carboxylate is immobilized and can interact with the side chain guanidinium group of Arg 94, as described above.

Variation in type II receptor binding

Residues structurally equivalent to ecT β R2 interfacial residues Leu 27, Phe 30, Thr 51 and Glu 75 have been converted to Ala residues by *in vitro* mutagenesis in ActR2, the mouse activin type II receptor. However, these mutations demonstrate no apparent effect on activin A binding¹⁵. Conversely, Ala mutations at positions Phe 42, Trp 60 and Phe 83 in ActR2 abrogate activin A binding¹⁵ but have no apparent effect on TGF- β 3 binding when the structurally equivalent Val 62, His 79 and Met 112 residues are mutated in ecT β R2 (data not shown). The most probable change in structure underlying these differences is the loop 4 insertion in T β R2 (Fig. 1b). *In vitro* mutagenesis of BMP-2 and analyses of its type II receptor ectodomain (ecBR2) binding properties demonstrate that ecBR2 interacts with BMP-2 by contacting amino acids Ala 34, His 39, Ser 88, Leu 90 and Leu 100 (ref. 21). These residues form a contiguous epitope, designated the knuckle, which maps to the convex surface of BMP-2, near the tips of fingers and opposite the ecT β R2 binding site in TGF- β 3 (Fig. 1c). Thus, ecBR2 binds BMP-2 through structural determinants distinct from the ecT β R2 epitope in TGF- β 3 (Figs 1c, 2b). These observations are consistent with the idea that type II receptors play the dominant role in determining the specificity of signaling complex assembly⁹ and may explain their relaxed sequence conservation across the superfamily (Fig. 2a).

Implications for receptor assembly

The recruitment of T β R1 by the (T β R2)₂-TGF- β complex was proposed to occur through either an allosteric or a cooperative binding mechanism⁸. In the allosteric model, the binding of T β R2 to TGF- β induces a conformational change in the ligand, exposing the T β R1 binding epitope. No direct T β R1–T β R2 interactions are required. Although the ecT β R2-bound form of

TGF- β 3 presented here (the open conformation) demonstrates a large conformational change relative to its uncomplexed form⁴ (the closed conformation), how ecT β R2 binding might cause the observed alterations in TGF- β 3 is unclear because ecT β R2 binds TGF- β 3 through the tips of the fingers at a position well removed from the homodimer interface. Furthermore, direct perturbations induced by ecT β R2 binding are minor (Fig. 1c) and occur in a region of the molecule that is highly flexible in the unbound state⁴. The open conformation of TGF- β 3 in the crystal structure of the complex is also incompatible with the probable mode of T β R1 binding suggested on the basis of the (ecBR1A)₂-BMP-2 crystal structure¹⁴. ecBR1A binds BMP-2 through a contiguous epitope, designated the wrist epitope, that consists of residues from both BMP-2 monomers — the same region that is disrupted and disordered in the ecT β R2-bound form of TGF- β 3 (Fig. 1c).

In the cooperative model of signaling complex assembly, T β R1 binds the (ecT β R2)₂-TGF- β 3 complex through an extended surface comprised of TGF- β 3 and one or more domains of T β R2 (ref. 8). Consistent with such a model, we note that the mode of TGF- β 3-binding to ecT β R2 is compatible with the canonical closed conformation of monomers in free TGF- β 3 and that the disordered region in the complex corresponds to the same region found to be conformationally labile in free TGF- β 3. Thus, ecT β R2-bound TGF- β 3 can probably also assume the closed conformation. If this were true, then T β R1 would be able to bind the T β R2-bound form of TGF- β 3 in the manner predicted by the (ecBR1A)₂-BMP-2 crystal structure, and the extracellular domains of the type I and type II receptors would be positioned adjacent to one another on the ligand surface. A model depicting this mode of assembly (Fig. 4) has been constructed by positioning the ecT β R2 domain into the crystal structure of the (ecBR1A)₂-BMP-2 complex¹⁴ at a position structurally equivalent to that observed when bound to TGF- β 3. In this binding mode, only minor steric clashes occur between components, and there is significant contact between the type I and type II receptor extracellular domains (449 Å² per polypeptide chain for the (ecT β R2)₂-(ecBR1A)₂-BMP-2 complex), which could facilitate cooperative receptor assembly.

In addition, the ecBR1A and ecT β R2 C-termini are oriented in a manner consistent with assembly of the signaling complex on the membrane surface. In the (ecT β R2)₂-(ecBR1A)₂-BMP-2 model, Phe 85 from ecBR1A, an invariant residue in type I receptors of the superfamily, packs neatly next to Ile 53 of ecT β R2 within the structurally conserved hydrophobic cavity formed by BMP-2 residues Trp 28, Trp 31, Tyr 91 and Tyr 103. Previously characterized interactions that might further stabilize such a complex and also be required for activation include heteromeric interactions between the transmembrane or cytoplasmic domains of the type I and type II receptors^{22,23} and

Table 1 Crystallographic data, phasing and refinement of the (ecTβR2)₂TGF-β3 complex

	Native	Platinum	Osmium	Iridium
λ (Å)	1.1000	1.0719	1.1301	1.1053
Number of observations	154,181	119,008	121,044	118,108
Number of unique reflections	29,006	12,516	14,673	14,635
Resolution range (Å) ¹	100–2.15 (2.23–2.15)	100–2.8 (2.87–2.80)	100–2.7 (2.76–2.70)	100–2.7 (2.76–2.70)
Completeness (%) ¹	99.8 (98.8)	93.8 (88.4)	99.9 (100.0)	99.8 (99.4)
R _{sym} (on I) (%) ^{1,2}	5.8 (53.3)	6.9 (40.7)	6.1 (21.7)	5.0 (19.0)
R _{iso} (%) ³		33.9	17.3	19.0
Phasing				
Number of sites		3	2	3
d _{min} (Å)	4.00			
Phasing power (centric / acentric) ⁴				
Isomorphous		0.83 / 0.98	0.92 / 1.08	0.77 / 0.85
Anomalous		– / 1.14	– / 1.19	– / 1.18
Refinement				
Resolution range (Å)	26–2.15	R.m.s. deviation		
R _{cryst} (%) ⁵	20.1	Bonds (Å)	0.014	
R _{free} (%) ⁶	21.5	Angles (°)	1.90	
F / σ F	>0	Number of molecules		
		Protein	1,494	
		Water	163	

¹The numbers in parentheses correspond to the last shell.

² $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity of multiple symmetry-related observations of that reflection.

³ $R_{\text{iso}} = \sum ||F_{\text{ph}}| - |F_{\text{p}}|| / \sum |F_{\text{p}}|$, where $|F_{\text{p}}|$ is the protein structure factor amplitude and $|F_{\text{ph}}|$ is the heavy-atom derivative structure factor amplitude.

⁴Phasing power = (r.m.s. $|F_{\text{h}}|$) / (r.m.s. E), where $|F_{\text{h}}|$ is the heavy-atom structure factor amplitude and E is the residual lack of closure error.

⁵ $R_{\text{cryst}} = \sum ||F_{\text{o,p}}| - |F_{\text{c,p}}|| / \sum |F_{\text{o,p}}|$.

⁶ $R_{\text{free}} = \sum ||F_{\text{o,p}}| - |F_{\text{c,p}}|| / \sum |F_{\text{o,p}}|$, where $|F_{\text{o,p}}|$ is from a test set not used in the structural refinement (2,002 reflections).

homomeric interactions between the cytoplasmic domains of the type I receptors²⁴.

The cooperative assembly mechanism is attractive because this type of interaction is noted among other cytokines, such as human growth hormone²⁵ and human prolactin²⁶, which also initiate the ordered sequential assembly of their signaling receptors. A cooperative assembly mechanism might also explain differences with which other ligands of the superfamily induce the assembly of their signaling receptors. Considering BMP-2, ecBR1A and ecBR2 for cooperative binding is interesting because the ecBR2 binding site on BMP-2 maps to the knuckle epitope on the convex surface ~6 Å from the tips of fingers 3 and 4, opposite the experimentally identified binding site for ecBR1A¹⁴. Thus, the (ecBR2)₂–(ecBR1A)₂–BMP-2 complex anticipated on the basis of the (ecBR1A)₂–BMP-2 crystal structure¹⁴ and the BMP-2 mutagenesis data²¹ would be expected to differ substantially from the (ecTβR2)₂–(ecTβR1)₂–TGF-β3 complex with respect to the relative orientation of the type I and type II extracellular domains and the extent of the contact surfaces between them. Relative to the TGF-β system, the diminished contact surface predicted between the extracellular domains of the BMP receptors might account for the observation that BMP-2 binds independently to both its type I and type II receptors^{27,28}, whereas TGF-β does not. This could also explain the marked interdependence with which TGF-β induces the assembly of its signaling receptors and the lack thereof for the BMPs⁹.

In summary, we have structurally defined the specificity of interaction between ecTβR2 and TGF-β3. Comparison of the crystallographic TβR2–TGF-β3 interface with either the activin-binding site on ecActR2 or the ecBR2-binding site on BMP-2 suggests there is significant structural diversity in the manner by

which type II receptors of the TGF-β superfamily interact with their cognate ligands. The validity of the cooperative assembly model presented here awaits the direct demonstration of such interactions through structural analysis of a heterotetramer consisting of homodimeric ligand and the extracellular domains of two type I and two type II receptors.

Methods

Protein expression and crystallization. Recombinant human ecTβR2 was obtained by *Escherichia coli* expression and refolding as described²⁹. *E. coli* recombinant human TGF-β3, kindly provided by OSI Pharmaceuticals, was further purified using reverse phase HPLC³⁰. (ecTβR2)₂–TGF-β3 complexes were formed by combining TGF-β3 and ecTβR2 in a 2.5:1 molar ratio in 25 mM glycine, pH 9.0. The complex was purified by gel filtration chromatography using 25 mM 2-(N-morpholino)ethanesulfonic acid (MES), pH 6.5, as the buffer. Crystals grew in space group R32 with unit cell parameters $a = b = 114.1$ Å and $c = 209.4$ Å (hexagonal setting) from hanging drops containing equal volumes (1–2 μl) of the protein complex at ~10 mg ml^{−1} and the reservoir solution (20% (v/v) 2-methyl-2,4-pentanediol (MPD) and 100 mM citrate, pH 4.0). The final pH of the freshly mixed crystallization solution was ~4.5.

Data collection, structure determination and refinement. All crystals were flash-cooled in liquid nitrogen before X-ray data collection. Diffraction data from native and derivative crystals were obtained at the NSLS beamline X8-C. The crystal-to-detector distance was 150 mm, and the oscillation angle was 1°. For each derivative data set, the X-ray wavelength was tuned to the absorption maximum for the heavy atom. Diffraction data were processed with the DENZO/SCALEPACK suite³¹. MIRAS phasing to 4.0 Å in SOLVE³² yielded an overall figure of merit of 0.63. Solvent flattening, histogram matching and phase extension to 3.0 Å in DM³³ produced readily interpretable electron density maps. The molecular two-fold axis of the TGF-β3 ligand is coincident with crystallographic two-fold axes, and the asymmetric unit contains one TGF-β3 and one

ecTβR2 monomer. The crystals have an open lattice and 75% solvent content ($V_m = 5.0$). Model building and manual readjustment were performed in O³⁴. Initial stages of refinement were accomplished in X-PLOR³⁵, and CNS³⁶ was used in the final stages. R_{free} was monitored in both refinement programs using identical test sets. Water molecules were introduced late in the refinement process where indicated by suitable 3 σ difference electron density and reasonable hydrogen bond geometry. Of all the non-Gly/Pro residues, 85% lie in most favored and none fall in disallowed regions of the Ramachandran plot.

Structure analysis and figure preparation. Solvent accessible surface areas were calculated with AREAMOL³³ using a probe radius of 1.4 Å. Structural alignments were performed using ALIGN³⁷. Figs 1a,c, 3a,b, 4 were prepared using MOLSCRIPT³⁸, BOBSCRIPT³⁹ and POV-Ray⁴⁰. Fig. 3c was prepared using GRASP⁴¹.

Coordinates. Coordinates of the structure have been deposited in the Protein Data Bank (accession code 1KTZ).

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Competing interests statement

The authors declare that they have no competing financial interests.

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