Review

Structural studies of the TGF-βs and their receptors – insights into evolution of the TGF-β superfamily

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1. Introduction

Transforming growth factor beta isoforms, TGF-β1, -β2, and -β3, are small secreted homodimeric signaling proteins. They are present only in vertebrates and are required for the proper development of several organs and tissues, including the heart, lungs, eyes, and palate [1–5]. They also perform functions essential for the long-term survival of humans and other higher vertebrates, including regulation of the adaptive immune system [6] and coordination of wound healing [7]. The dysregulation of the TGF-β pathway leads to a number of human diseases and disorders, including tissue fibrosis [8] and cancer [9–11], demonstrating the essential roles the TGF-β isoforms have in vivo.

TGF-βs belong to a diversified family of signaling proteins, known as the TGF-β superfamily. The other members of the superfamily include the bone morphogenetic proteins (BMPs) which regulate embryonic patterning [12], the closely related growth and differentiation factors (GDFs) which regulate cartilage and skeletal development [13], the activins (Acts) and inhibins (Inhs) which regulate the release of pituitary hormones [14,15], and others, such as Müllerian inhibiting substance (MIS) that regulate sex determination during embryonic development. The proteins of the TGF-β superfamily share the same dimeric structure in which the central 3–1/2 turn helix of one monomer packs against the concave surface formed by the β-strands of the other monomer (Fig. 1). The majority of family members are further stabilized by an inter-chain disulfide bond that links the monomers together.

There are homologs of the BMPs in worms and flies [16], the activins/inhibins in flies, but not in worms [17], and TGF-βs in neither, indicating that the first evolved members of the superfamily were the BMPs/GDFs and that these diverged first into the activins/inhibins and later into the TGF-βs. This is further supported by phylogenetic analysis of vertebrate superfamily members that show they progressively diverge from the many different BMP/GDFs, with activins/inhibins being close intermediates and the TGF-βs and other proteins that play essential roles in vertebrate development, including MIS and Lefty, being the most divergent (Fig. 2).

TGF-βs transduce their signals by binding and bringing together two single-pass transmembrane receptor kinases into a kinase-active heterotetramer [18,19]. These receptors, known as the TGF-β type I and type II receptors, or TβR-I and TβR-II, are structurally similar and include small disulfide-rich ectodomains that adopt a three-finger toxin fold (~120 residues) [20], single-spanning transmembrane domains (~30 residues), and cytoplasmic serine-threonine kinase domains (~400 residues) (Fig. 3). The assembly of TβR-I and TβR-II into a heterotetramer triggers a transphosphorylation cascade that begins with the TβR-II-mediated activation of the TβR-I kinase [21] and is propagated by the TβR-I kinase to intracellular effectors, including both the canonical receptor-
mediated Smad proteins (R-Smads) [22,23] and non-Smads [24–31]. This manner of signaling, including the requirement for a type I and type II receptor and the type II receptor-mediated activation of the type I receptor, is shared by all proteins of the superfamily.

The proteins of the superfamily can nevertheless be divided into two phylogenetic clades based on the type I receptors they bind and the Smad proteins they activate – the more recently evolved members of the superfamily, which includes the TGF-βs, activins, GDF-8, GDF-9, GDF-11, BMP-3, and nodal, bind and signal through type I receptors that couple to and activate R-Smads 2, 3 (Table 1). The more distantly related proteins of the superfamily, which includes BMP-2, -4, -5, -6, -7, -8, -9, and -10 and GDF-1, -3, -5, -6, and -7, in contrast, bind and signal through type I receptors that couple to and activate R-Smads 1, 5, and 8 (Table 1). The two subclasses of R-Smads, R-Smads 2, 3 and R-Smads 1, 5, and 8, upon association with the co-mediator Smad, Smad4, assemble distinct transcriptional complexes and thus activate distinct subsets of genes [22]. The TGF-βs are further distinguished from other members of the superfamily with regard to their restrictive manner of receptor binding – thus with one minor exception discussed in greater detail below, the only receptors that have been reported to bind and transduce signals for the TGF-βs are TβR-I and TβR-II. This stands in contrast to the activins, which promiscuously bind and signal through at least two different type I receptors (ActR-Ib and TβR-I) and two different type II receptors (ActR-II and ActR-IIb), and some BMPs and GDFs, which promiscuously bind and signal through as many as three type I receptors (BMPR-Ia, BMPR-Ib, and ActR-I) and three type II receptors (ActR-II, ActR-IIb, and BMPR-II) (Table 1).

The activation of distinct subsets of effector proteins by the two clades of the superfamily represents an important evolutionary adaptation that has allowed the superfamily to diversify. The coupling of proteins of the two clades to specific subsets of R-Smads necessitates, however, that the receptors bind both the signaling...
proteins and R-Smads with high specificity. The molecular underpinnings of the receptor-R-Smad binding involving the so-called L45 loop of the type I kinase[32] and the L3 loop of the Smad MH2 domain [33] have been understood for more than a decade, while those between the signaling proteins and receptors have only become apparent in the past few years. These new findings will be discussed in the following section with an emphasis on the restrictive manner by which the TGF-βs bind and assemble their receptors into signaling complex and how this differs from the more distantly related BMPs and GDFs, such as BMP-2, BMP-4, and BMP-7, which promiscuously bind multiple type I and type II receptors.

2. TGF-βs and BMPs bind and assemble their receptors in a distinct manner

The mechanisms by which proteins of the superfamily bind and assemble their receptors into signaling complexes were initially probed by affinity labeling. The most elegant studies were those reported for TGF-β using receptor-deficient mink lung epithelial cells [34]. These studies showed that TGF-β and TβR-II together crosslink to TβR-I, but TGF-β alone does not. This pattern was also reported for activin [35], leading to the proposal that TGF-βs and activins assemble their receptors in an ordered manner, first by binding their type II receptor and then by recruiting their type I receptor [36]. The BMPs and GDFs, in contrast, exhibited a much more heterogenous pattern of crosslinking, with some crosslinking to their receptors in a stepwise manner, while others crosslinked their receptors when expressed together, but not when either receptor was expressed alone [37–43]. These findings, together with differences in the promiscuity of receptor binding, hinted that the TGF-βs/activins and BMPs/GDFs might differ in the manner by which they bind and assemble their receptors into signaling complexes.

These differences in receptor binding have been borne out by the structures of the TGF-β and BMP type I type II receptor ternary complexes determined over the past several years. The structure of the TβR-II kinase domain has not been reported – that shown corresponds to ActR-IIb (PDB entry 2QLU).

![Diagram of TGF-β type I and type II receptors](image)

**Fig. 3.** The TGF-β type I and type II receptors, TβR-I and TβR-II. The extracellular domains (ECDs) of TβR-I and TβR-II are small (101 and 136 residues, respectively), heavily disulfide-bonded (five and six disulfides, respectively), and adopt a three-finger toxin fold (F1, F2, and F3 designate the three fingers of the receptor three-finger toxin fold). The transmembrane domain (TMDs) and cytoplasmic serine-threonine kinase domains (S/TKD) are also shown. The type I receptor includes a ~20 amino acid juxtamembrane glycine-serine rich regulatory domain, known as the GS box (GS, purple). The structurally disordered residues between the structured portion of the ectodomain or kinase domain and the transmembrane domain are shown by dashed lines (14, 22, 8, and 26 residues for the TβR-I ecto, TβR-I kinase, TβR-II ecto, and TβR-II kinase domains, respectively). The TβR-I ECD, TβR-II ECD, and TβR-I kinase structures are from PDB entries 2PJY, 1M9Z, and 1IAS, respectively. The structure of the TβR-II kinase domain has not been reported – that shown corresponds to ActR-IIb (PDB entry 2QLU).

Table 1

<table>
<thead>
<tr>
<th>Signaling protein</th>
<th>Type I receptor(^a)</th>
<th>Type II receptor</th>
<th>Smad</th>
</tr>
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<tbody>
<tr>
<td>TGF-βs</td>
<td>TβR-I (Alk5)</td>
<td>TβR-II</td>
<td>Smad2, 3</td>
</tr>
<tr>
<td>Activin</td>
<td>ActRI-b (Alk4)</td>
<td>TβR-I (Alk5)</td>
<td>Smad2, 3</td>
</tr>
<tr>
<td>GDF-8 (Myostatin)</td>
<td>ActR-Ib (Alk4), TβR-I (Alk5)</td>
<td>ActR-II, ActR-Ib</td>
<td>Smad2, 3</td>
</tr>
<tr>
<td>Nodal</td>
<td>ActR-Ib (Alk4), ActR-II (Alk7)</td>
<td>ActR-II, ActR-Ib</td>
<td>Smad2, 3</td>
</tr>
<tr>
<td>BMPs, GDFs</td>
<td>Alk1, ActR-II (Alk2)</td>
<td>ActR-II, ActR-Ib BMPR-II</td>
<td>Smad1, 5, 8</td>
</tr>
<tr>
<td>MIS</td>
<td>BMPR-1a (Alk3)</td>
<td>BMPR-Ib (Alk6)</td>
<td>MISR-II</td>
</tr>
</tbody>
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\(^a\) Type I receptors of the TGF-β superfamily were initially designated as activin-like kinases, or Alks, but many have since be named according to the major subfamilies of signaling proteins that they serve.
structures of BMP-2 and BMP-7 bound to the ectodomains of the BMP type I and type II receptors, BMPR-Ia and ActR-II [44,45] and was later confirmed when the structures of two closely related BMP ternary complexes, BMP-2:BMPR-Ia:ActR-II and BMP-2:BMPR-Ia:ActR-IIb, were determined [46,47]. The structure of the TGF-β ternary complex was initially inferred based on the structure of TGF-β3 bound to the TβR-II ectodomain and a hypothetical positioning of TβR-I based on BMPR-Ia [48], but this was revised after the structures of the TGF-β3:TβR-II:TβR-I and TGF-β1:TβR-II:TβR-I complexes were determined and it was shown that TβR-I binds differently than BMPR-Ia [49,50].

The structures of the ternary complexes show that although the signaling proteins and receptors of the BMP and TGF-β subfamilies share the same overall fold (Figs. 2 and 4a, respectively), they nevertheless bind their receptors in a distinct manner (Fig. 4b and c). The BMP type I and type II receptors bind to the “wrist” and “knuckle” epitopes, respectively, and do not contact one another, while the TGF-β type I and type II receptors bind to the underside of the “fingers” and to the “fingertips”, respectively, and have extensive contact. The direct contact between the type I and type II receptors in the TGF-β complex, but not the BMP, suggested that TβR-I is recruited by binding both TGF-β and TβR-II. The importance of direct receptor–receptor contact for the recruitment of TβR-I was initially confirmed by binding studies with the purified receptor ectodomains [49,51]. These studies showed that the TβR-I ectodomain potentiates the binding of TβR-II ectodomain by 500-fold or more, whereas similar experiments performed with BMPs and the BMP receptor ectodomains yielded much more modest effects (potentiation of binding of one receptor by the other ranging from 0-fold [47] to 3- to 4-fold) [44]. The importance of the direct receptor–receptor contact for recruitment of TβR-I into the TGF-β receptor complex has since been confirmed by
substituting residues in TβR-II that directly contact TβR-I in the structure of the ternary complex [49]. These studies, performed with both the purified TβR-I and TβR-II ectodomains and the membrane-bound form of the receptors in stably transfected cell lines, showed that the substitutions had no effect on the ability of TβR-II to bind TGF-β, but did impair the ability of the TGF-β: TβR-II complex to bind and recruit TβR-I. Thus, the receptor–receptor contacts identified in vitro through crystallography and accompanying binding studies are both required for TGF-β signaling and underlie the pronounced stepwise manner by which TGF-β binds and assembles its receptors in cells.

3. Structural adaptations that enable distinctive modes of receptor binding

The TGF-β and BMP ternary complexes differ in the manner by which both receptor types bind (Figs. 5a, 6a). The differences are especially pronounced for the type II receptor – BMP type II receptors utilize the concave surface of their β-sheet to complement the convex surface of the knuckle epitope of the BMP (Fig. 5a and c), while the TGF-β type II receptor inserts one of its edge β-strands into the cleft between the TGF-β fingertips (Fig. 5f). Though the differences are less pronounced, the type I receptors also bind differently – BMP type I receptors bind to the wrist and have extensive contact with both BMP monomers (Fig. 6c), while the TGF-β type I receptor moves away from the wrist toward the fingertips where it contacts TβR-II, the TGF-β monomer to which TβR-II is bound (so-called “A” monomer), and to a limited extent, the adjacent monomer (so-called “B” monomer) (Fig. 6f). These two binding modes are also distinguished by...
the nature of the interactions, with the BMP type I receptor binding through a large interface dominated by hydrophobic interactions (Fig. 6c) and the TGF-β type I receptor binding through a smaller interface with a combination of hydrophobic and electrostatic interactions (Fig. 6f).

The structures of the receptor complexes [44–47,49,50], together with accompanying functional studies [52,53], show that the repositioning of the receptors is driven by relatively minor, but important changes in the loop regions of the receptors. The repositioning of the type II receptor is driven by an extension of the loop that connects β-strands 4 and 5 of the receptor three-finger toxin fold (Fig. 5b). This loop is short and oriented away from the concave surface of the β-sheet in the BMP type II receptor (Fig. 5b, cyan), while in the TGF-β type II receptor it is lengthened by seven residues and folds back onto the concave surface of the sheet (Fig. 5b, red). The loop in TβR-II is rigidly held in place by hydrophobic residues that interdigitate with hydrophobic residues on the underlying sheet (Fig. 5b and d) [54]. The extended loop in TβR-II precludes binding in a BMP-like manner since it sterically overlaps with the fingers when bound on the knuckle (Fig. 5d).

The repositioning of the type I receptor is also driven by an extension of the loop that connects β-strands 4 and 5. The loop is structurally disordered in the unbound of the BMP type I receptor, BMPR-la [55], but undergoes a disorder-to-order transition to form
a short helix upon binding [45–47]. This helix includes an exposed phenylalanine residue, Phe85, that binds in the hydrophobic pocket formed by Trp28 and Trp31 on the wrist region of the BMP dimer (Fig. 6e). The β4–β5 loop, in contrast, is structurally ordered in the unbound form of the TGF-β type I receptor, TßR-I, and in addition to the central helix, also includes a five-residue ‘pre-helix’ extension in the N-terminal portion of the loop [53] (Fig. 6e). The pre-helix extension adopts a tight turn with a characteristic N-terminal cis proline [49,50,53] and precludes binding a BMP-like manner since it is rigid [53] and sterically overlaps with the wrist region of the BMP “A” monomer (Fig. 6d).

The steric overlap caused by the rigid extensions of β4–β5 loop leads to entirely new interfaces for TßR-I bound to TGF-β3 and for TßR-I bound to the TGF-β3:TßR-II complex. These interfaces are illustrated in Figs. 5f and 6f and as shown the interactions that enable binding – for TßR-II a central hydrophobic cluster with two hydrogen-bonded ion pairs at the periphery, and for TßR-I a combination of hydrophobic and electrostatic interactions orchestrated by the pre-helix extension as it binds deeply in the cleft between the TGF-β “A” monomer and the adjacent bound TßR-II – are generally not possible with the corresponding BMP receptors (Figs. 5e and 6e). The lack of stabilizing interactions is to be expected since there is no evolutionary pressure to optimize these interactions in the BMP system – and in fact negative selection may be at work since binding of the TGF-βs by BMP receptors would lead to inappropriate TGF-β signaling, which would presumably have adverse effects in vivo.

The repositioning of the receptors appears to have been important for increasing the range of specificity within the superfamily. Thus, little or no sharing of receptors has been observed among signaling proteins that bind their type I and type II receptors through distinct interfaces, while significant sharing of receptors has been observed among proteins of the superfamily that bind their receptors using the same interface. The former is illustrated by the TGF-βs and the distantly related BMPs and GDFs which bind their type I and type II receptors in a distinctive manner and do not share receptors. The latter is illustrated by the distantly BMPs and GDFs which bind their type I and type II receptors in the same overall manner [44,45,47,56] and which are well known to share their receptors [57]. This is further illustrated by the structures that have been reported for the activins bound to their type II receptors, ActR-II and ActR-Iib [58,59]. The activin A:ActR-II and activin A:ActR-Iib structures show that activins also bind their type II receptors in the same overall as the BMPs and GDFs, consistent with the sharing of ActR-II and ActR-Iib not only among the many different BMPs and GDFs, but also among the activins.

The segregation of the activities of the TGF-βs and the distantly related BMPs and GDFs is directly dependent upon the specificity with which these classes of proteins bind their type I receptors. The high specificity with which TGF-βs bind TßR-I is likely dependent upon the evolutionary modifications that gave rise to the alternative mode of type I receptor binding. The evolutionary modifications that gave rise to the alternative mode of type II receptor binding however also likely contributes to the specificity as this creates the interface between TßR-I and TßR-II. The TßR-I:TßR-II interface adds an important additional restraint that must be satisfied to enable type I receptor binding, increasing specificity even further. The distinctive manner by which the TGF-βs bind both their type I and type II receptors is therefore likely responsible for TGF-β’s high specificity for binding TßR-I and in turn for segregating the activities of the TGF-βs from the other proteins of the superfamily, particularly the distantly related BMPs and GDFs, but also more closely related proteins such activins, GDF-8, and nodal. This may be important for allowing the TGF-βs to carry out their indispensable functions – coordinating wound repair and regulating the adaptive immune system – without interference from the many other proteins of the superfamily.

Though the functional data and structural data amply demonstrate that TGF-β is highly specific for binding and recruiting TßR-I, other type I receptors may be able to bind and signal in place of TßR-I. The most extensively studied of these is Alk1, which is expressed at high levels in many types of endothelial cells and which has been reported to form a mixed receptor complex with TGF-β, TßR-II, and TßR-I [60]. This complex is reported to activate Smads 1, 5, and 8, in addition to Smads 2 and 3, and has been proposed to underlie TGF-βs opposing effects on the migration of endothelial cells. This ‘lateral signaling’ phenomenon has also been shown to occur in the context of several different normal and transformed cell lines with the type I receptors ActR-I and BMPR-Ia [61,62]. The fact that these type I receptors are capable of substituting for TßR-I and transducing signals in response to TGF-β, albeit with significantly reduced efficiency, may reflect the ability of these type I receptors to transiently bind into the space between TßR-II and TGF-β, become phosphorylated by TßR-II, and signal. This presumes that these receptors retain sufficient affinity to bind even though they lack the critical pre-helix extension. Though further experimentation is required, this seems plausible given recent studies that have shown that substitution of the proline residue on the N-terminal end of the TßR-I pre-helix extension significantly impairs its recruitment into the TGF-β receptor complex, but does not entirely prevent it [53]. Thus, while TGF-β has clearly evolved to recruit and signal through TßR-I, there may nevertheless be circumstances, such as when levels of receptors such as Alk1 are increased, that enable a small proportion of the complexes formed to include a non-canonical type I receptor, such as Alk1.

4. Activin as an evolutionary intermediate?

The distinct modes of type I and type II receptor binding for the TGF-βs raise the question as to how these two binding modes evolved – did these two binding modes simultaneously evolve during a period of rapid diversification, or did they evolve in a stepwise manner through an intermediate with a mixed mode of receptor binding? Though this question cannot be directly addressed, it is tempting to speculate that the new modes of binding arose in a stepwise manner, first by evolution of a new mode of type I receptor binding and later by a new mode of type II receptor binding. This hypothesis is based on our current understanding of how activins bind their receptors. The activins both share their type II receptors with the BMPs and bind their type II receptors in the same overall manner as the BMPs [58,59], unambiguously demonstrating that they bind their type II receptors in a BMP-like manner. The activin type I receptor, ActR-Ib, has not yet been structurally characterized, yet it shares several characteristics with TßR-I, which also serves as a type I receptor for activins. The first is that it represents the only other known type I receptor of the superfamily that includes a ‘pre-helix’ extension within the β4–β5 loop. The extension is similarly positioned in the N-terminal portion of the loop and it has a similar sequence, including the proline residues on the N- and C-terminal ends shown to be critical for recruitment of TßR-I into the TGF-β receptor complex (loop sequence is PAGKP in ActR-Ib and PRDRP in TßR-I) [53]. The second is that a systematic study of surface residues of ActR-Ib potentially involved in receptor binding showed that the residues that most strongly perturb recruitment of ActR-Ib into the activin receptor complex map to the pre-helix extension, not to the region on the concave surface of the β-sheet known to be important for BMP type I receptor binding [63]. These findings demonstrate the importance of ActR-Ib’s pre-helix extension for binding and tentatively supports the hypothesis that activins bind their type I receptor in a TGF-β-like manner. The logical extension of this is that all the
proteins of the superfamily that bind and signal through type I receptors that couple to and activate R-Smads 2, 3 bind their type I receptors in a TGF-β-like manner – whether this is true or not will require the determination of structures of proteins such as activins, GDF-8, and nodal bound to type I receptors, such as ActR-Ib and TβR-II.

This mixed mode of receptor binding, if demonstrated through direct structural analysis of the activin A:ActR-II:ActR-Ib or activin A:ActR-Iib:ActR-Ib complex, would position the type I and type II receptors such that they have a gap of 8–10 Å between them, necessitating a mechanism other than receptor–receptor contact as underlying ordered assembly. There are two alternative mechanisms by which this might occur – both involve potentiation of type I receptor binding by the type II receptor, but by different mechanisms. The first mechanism suggests that the membrane-bound form of ActR-II or ActR-Iib induces a conformational change in activin upon binding, thus potentiating the binding of ActR-Ib [58]. This mechanism was suggested based on the ‘open’ structures of activin A that have been reported (see below), but has not been evaluated experimentally by demonstrating either a type II receptor-dependent conformational change in activin or a type II receptor–dependent potentiation of type I receptor binding. The second mechanism suggests that the high affinity type II receptors, ActR-II and ActR-Iib, which bind activins with near nanomolar affinity [58,59], concentrate activin on the cell surface and potentiate the binding of the low affinity receptor, ActR-Ib. This mechanism has previously been shown to be important for the ordered assembly observed with some BMPs, such as BMP-7, which binds the type II receptor ActR-II with high affinity and in turn recruits the lower affinity type I receptor BMPR-la [64]. Thus, membrane concentration and reduction of dimensionality might also be important for ordered receptor assembly by the activins, though this has also not been evaluated experimentally.

5. Influence of distinct modes of receptor binding on the structure of the signaling proteins

The structures shown in Fig. 1 are representative of a number of superfamly proteins, including the first protein of the superfamily to be structurally characterized, TGF-β2 [65,66], as well as a number of others since determined, including TGF-β1 [67], TGF-β3 [68], BMP-2 [69], BMP-3 [70], BMP-6 [70], BMP-7 [71], BMP-9 [72], and GDF-5 [56,73]. The presumption that all proteins of the superfamily adopt such ‘closed’ dimers was challenged when the structure of TGF-β3 bound to TβR-II was reported [48]. TGF-β3 was shown to adopt a non-canonical ‘open’ dimer, characterized by a rotation of the monomers away from one another and a structural disordering of the interfacial α-helix (Fig. 7a). The presumption of a common ‘closed’ dimer was further challenged when activin A was crystallized with ActR-Iib [59] and was found to adopt an open state with many of the same characteristics, including a non-canonical arrangement of the monomers and a disordered helix (Fig. 7b). There has been one subsequent report indicating that TGF-β3 adopts an open state [74] (n.b. this paper reports two TGF-β3 structures – the primary focus of the paper, the antibody-bound form is found to be ‘closed’, while the unbound form, mentioned only in the text, is found to be ‘open’). Two subsequent reports indicating the activin A adopts an open state [58,75]. These structures while revealing similar, though not identical open forms, were determined by independent investigators under entirely different conditions – for example, the most recent structure of TGF-β3 was determined at pH 7 in the absence of TβR-II, whereas the previously reported structure was determined at pH 4 in the presence of TβR-II. This suggests the open forms are not a consequence of the conditions under which the structures were determined. This raises questions as to whether the open forms are peculiar to the TGF-βs and activins and whether such forms are functionally relevant.

Though there has been little progress made toward directly addressing these questions, it is nevertheless tempting to speculate that the open forms might be restricted to the TGF-βs and other proteins of the superfamily that bind their type I receptors in the same overall manner as TβR-I. The hypothesis is based on the TGF-β ternary complex structures [49,50] – in these structures TGF-β adopts the closed form, TβR-II is bound to residues from a single monomer, and TβR-I is bound such that it contacts TβR-II and the TGF-β A-monomer, but only to a limited extent to the adjacent B-monomer. The latter is illustrated in Fig. 7c where it is shown that there is a large water-filled cleft between TβR-I and the B-monomer in the structure of the TGF-β3:TβR-II:TβR-I complex. The limited importance of the B-monomer for TβR-I binding has been demonstrated by binding studies in which the Kd for recruitment of TβR-I by either a dimeric binary complex (TGF-β3 dimer bound to two equivalents of TβR-II) or a monomeric binary complex (TGF-β3 monomer, generated by substituting the cysteine that forms the inter-chain disulfide with serine, bound to one equivalent of TβR-II) was measured and found to be diminished only 10-fold [49,76]. Thus, disruption of the closed dimer by a rotation of the monomers away from one another as shown in Fig. 7c would be expected to diminish binding, but only to a limited extent. This stands in contrast to the much greater reduction in type I receptor binding affinity that would be expected by rotation of the BMP and GDF monomers away from one another. The BMP type I receptors have extensive contact with both monomers and thus a much greater reduction in type I receptor binding affinity is expected as the monomers rotate away from one another. This may account for the fact that all of the BMPs/GDFs that have been structurally characterized have been found in the closed form. Thus, TGF-βs and other proteins of the superfamily whose type I receptors bind with only limited contact with the “B” monomer (n.b. this may include the activins, as well as other closely related proteins such as GDF-8, GDF-9, BMP-3, and nodal) achieve only marginal increases in type I receptor binding affinity by maintaining a closed dimer – these proteins are therefore much more likely to be found in the open form. BMPs, GDFs and other proteins whose type I receptors bind with extensive contact to both monomers in contrast are likely to achieve much greater gains in type I receptor binding affinity by maintaining a closed dimer – these proteins are therefore much more likely to be found in the closed form.

There have been no studies yet reported linking the open and closed forms to the distinct functions of proteins of the superfamily – one possibility, especially if the effects of the open forms on receptor binding are minimal, is that they have no major functional relevance. There are however other functions that the open states might have acquired – for example, they might enable recognition by binding proteins that modulate activity or alternatively they might alter signaling due to a transient opening of the signaling protein in the context of the signaling complex.

6. Summary and future prospects

The structural studies reported to date have shown that the TGF-βs diverged from the BMPs and GDFs, the ancestral ligands of the superfamily, to bind and assemble their type I and type II receptors in a distinct manner. This diversification, with the type I and type II receptors directly contacting one another in the TGF-β receptor complex, but not the BMP, is likely important in preventing other ligands of the superfamily from interfering with the vital cellular processes that TGF-βs control [77]. There may,
Fig. 7. Structures of the open forms of TGF-β3 and activin A. (a) Structure of TGF-β3 in the canonical closed form in which the two monomers tightly pack against one another (left) and the open form in which the two monomers are only tethered together by the inter-chain disulfide and the central helix is structurally disordered (dashed lines, right) [48]. A single molecule of TβR-II bound to the fingertips is shown for reference (opening of the dimer does not disrupt the binding site for TβR-II). Opening of the TGF-β3 dimer occurs due to a 100° rotation around an axis parallel to the long dimension of TGF-β. (b) Structure of activin A in the canonical closed form in which the two monomers tightly pack against one another (left) and the open form in which the two monomers are only tethered together by the inter-chain disulfide and the central helix is structurally disordered (dashed lines, right) [59]. A single molecule of ActR-IIb bound to the knuckles is shown for reference (opening of the dimer does not disrupt the binding site for ActR-IIb). Opening of the activin A dimer occurs due to a 100° rotation of the two activin A monomers toward one another. (c) Structure of TβR-I:TβR-II:TGF-β3 complex showing that TβR-I mainly contacts TβR-II (green) and the TGF-β monomer to which TβR-II is bound (A monomer, magenta). TβR-I’s pre-helix extension, identified by a dashed black line, is shown to bind deeply in the cleft between TβR-II and the TGF-β3 A monomer. The large water-filled cleft between TβR-I (tan) and the TGF-β3 B-monomer (pink) is highlighted by the dashed red line.

References


