TGF-β Antagonists: Same Knot, but Different Hold

Andrew P. Hinck[1,*] and Tao Huang[2]
[1]Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900, USA
[2]Structural Biophysics Laboratory, National Cancer Institute, Frederick, MD 21702, USA
*Correspondence: hinck@uthscsa.edu
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In this issue of *Structure*, Nolan and colleagues present the structure of BMP antagonist, PRDC, which adopts a head-to-tail dimer with distinct structure and inhibitory mechanism compared to other dimeric antagonists of the TGF-β superfamily, such as noggin.

The transforming growth factor-beta (TGF-β) superfamily is comprised of a diversified family of secreted signaling proteins, with more than 30 members in humans and other vertebrates (Hinck, 2012). The proteins of the superfamily evolved as developmental factors responsible for embryonic patterning and morphogenesis in invertebrates, but have further evolved to regulate numerous extraembryonic functions as organisms have diversified. These include, but are not limited to, the regulation of bone and muscle mass by BMP-7 and GDF-8, regulation of gonadal function by the activins and inhibins, regulation of the adaptive immune system by the TGF-βs, and regulation of the differentiation of embryonic stem cells by activins and nodal. The proteins of the superfamily regulate hundreds of genes, and thus it is not surprising that new functions, such as the ability of BMPs to regulate the differentiation of cancer stem cells in cooperation with the secreted antagonist coco, are still being discovered (Gao et al., 2012).

TGF-βs, BMPs, GDFs, and other proteins of the superfamily are structurally similar, consisting of two extended monomers held together in most, but not all cases, by a single disulfide bond (Figure 1A) (Hinck, 2012). The monomers of all superfamily members include a cystine knot, which is formed by three disulfides, where the first and second bridge adjacent β strands, while a third passes through the eight residue ring formed by the first and second disulfide. The extended β sheet structure, together with the stabilizing cystine knot, is known as a growth factor fold. This fold is present in a number of other secreted signaling proteins, including nerve growth factor (NGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and others (Figure 1B). These signaling proteins are also active as disulfide-linked dimers, though the arrangement of monomers differs and is responsible for their signaling through distinct receptors and disparate activities (Figure 1A).

The proteins of the TGF-β superfamily signal by binding and bringing together two transmembrane receptors, known as receptor types I and II. The assembly of these receptors into heteromeric complexes leads to the activation of the type I receptor kinase, which in turn activates cytoplasmic effectors, known as Smads (Massagué et al., 2005). There are seven type I receptors and five type II receptors in most vertebrate species, and among these, the type I receptors couple to two different classes of Smads. The more recently evolved members of the superfamily, including the TGF-βs, activins, nodal, and some of the GDFs and BMPs (GDF-9, -11, and -15 and BMP-15), bind and signal through type I receptors that activate R-Smads 2 and 3, while the more distantly related GDFs (GDF-1, -3, -5, -7, and -10) and BMPs (BMP-2, -3, -4, -5, -6, -7, -8, -9, and -10) bind and signal through type I receptors that couple to and activate R-Smads 1, 5, and 8 (Hinck, 2012). This restricts the functional diversity that can be attained through intrinsic differences in signaling. The diversity of signaling is instead dependent upon the unique patterns with which the superfamily ligands are targeted to different cells and tissues and the context-dependent manner by which cells respond to activated Smads (Massagué and Wotton, 2000).

The targeting of superfamily signaling proteins is largely mediated by secreted antagonists, which bind the signaling proteins and block the receptor binding sites. The antagonists are structurally diverse, ranging from large multidomain proteins, such as follistatin and chordin, to smaller single domain proteins with a cystine knot growth factor fold, such as those of the differential screening-selected gene aberrative in neuroblastoma (DAN) family and noggin (Bragdon et al., 2011). The structural diversity of the antagonists stands in contrast to the signaling proteins and is thought to be responsible for the specificity of most toward a limited subset of signaling proteins. Though the secreted antagonists have vital roles targeting superfamily signaling proteins to specific cells and tissues, there is, at present, only a limited molecular understanding of their diverse molecular structures and inhibitory mechanisms (Cash et al., 2009; Groppe et al., 2002).

The focus of the Nolan et al. (2013; in this issue of *Structure*) discussion is the DAN family antagonist, protein related to DAN and cerberus, or PRDC. Although all nine members of the DAN family include a cystine-knot motif and adopt a growth factor fold, they differ significantly in their inhibitory potencies and the subset of signaling proteins they target. The most potent DAN family antagonists, DAN, PRDC, and Gremlin, are thought to only antagonize BMPs and other superfamily signaling proteins, while the least potent of the DAN family antagonists, SOST and USAG-1, also bind the coreceptor LRP5/6 to antagonize Wnt signaling. The only structural information available for the DAN family of antagonists is SOST, which includes an even number of cysteines and is monomeric (Figure 1B) (Veverka et al., 2009). This stands in contrast to PRDC, which, through prior studies, had been shown to form a highly stable noncovalent dimer even though it includes an odd

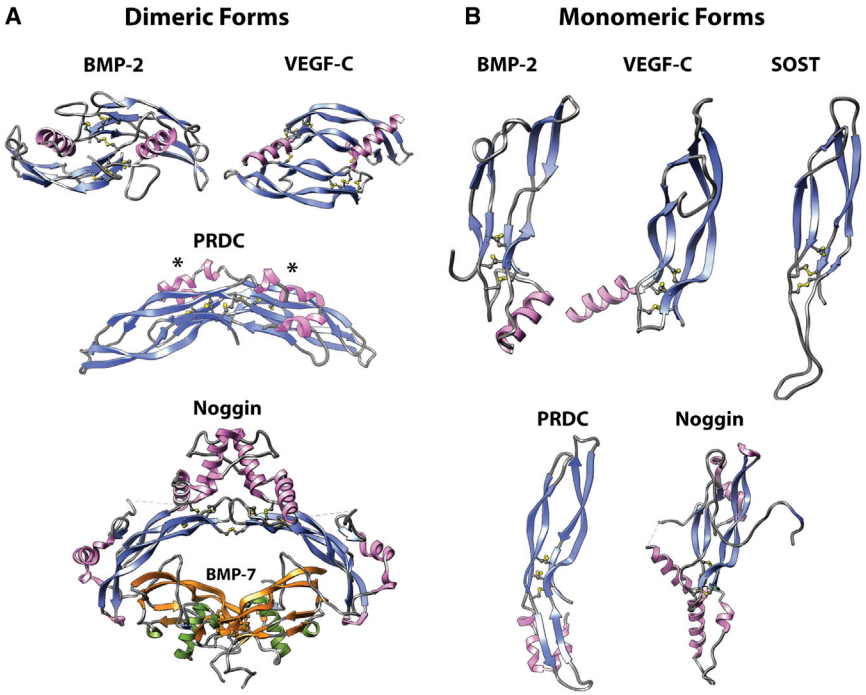

Figure 1. The Malleability of the Cystine Knot Growth Factor Fold

(A) Dimeric forms of the cystine-knotted signaling proteins, BMP-2 and VEGF-C, and the BMP antagonists, PRDC and noggin. Disulfide bonds that form the cystine knot, as well as those that form the interchain disulfide(s) in BMP-2, VEGF-C, and noggin are depicted using a ball-and-stick representation. Asterisks on the PRDC structure designate the BMP binding site as identified through site-directed mutagenesis and accompanying functional studies.

(B) Monomeric forms of the cystine-knotted signaling proteins and the BMP antagonists shown in (A) (shown also is the monomeric BMP antagonist SOST). Disulfide bonds that form the cystine knot are depicted using a ball-and-stick representation as in (A).

number of cysteines, including one (C120) that is positionally conserved with the cysteine that forms the interchain disulfide in TGF-βs and other proteins of the TGF-β superfamily (Kattamuri et al., 2012). The reason for this did not become apparent until the structure of PRDC was determined and it was shown that PRDC forms a head-to-tail dimer with an interface one-and-a-half times larger than the signaling proteins of the TGF-β superfamily and extensive hydrogen bonding between the exposed β strand of finger 2 (Figure 1A). This alternative manner of dimerization positions the monomers so that C120 is incapable of forming the interchain disulfide characteristic of most proteins of the TGF-β superfamily. The authors further showed that the residues of PRDC responsible for binding BMPs reside largely on the convex surface of the PRDC dimer in the cysteine-rich DAN domain. These findings suggest that PRDC achieves high affinity for BMP dimers due to multivalent binding (dimeric PRDC binding to dimeric BMPs). The more distantly related cystine-knot BMP antagonist noggin was also previously shown to bind BMPs with high affinity by forming a dimer, but this differs in two significant ways relative to PRDC. The first is that, unlike PRDC, noggin forms an unusual head-to-head dimer that is stabilized both by a single interchain disulfide and by the addition of several helical segments that pack against one another at the dimer interface (Figure 1A). The second is that noggin also forms an arch-like structure, but unlike PRDC, it uses its concave surface, together with an extended clamp-like structure, to nearly fully surround the signaling protein and block the type I and type II receptor binding sites.

The structure of PRDC is significant, because it shows how the same cystine-knotted growth factor fold can be modified to form two entirely distinct BMP antagonists. The finding that alternate arrangements of the cystine-knotted growth factor have given rise to distinct antagonists with distinct specificities is perhaps not surprising given the diversity of the dimeric structures among the different classes of cystine-knotted growth factors (TGF-β, PDGF, NGF, VEGF, etc.) and their binding to distinct receptors. The structure of PRDC nevertheless reiterates the malleability of this important structural motif and the many ways in which it has evolved to expand and diversify cell signaling. The future studies of other DAN family antagonists, such as gremlin, cerberus, coco, and others, therefore promise to offer plenty of additional surprises. We are looking forward to seeing what else this domain can do!

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