

Extracellular Regulations of TGF- β Signaling

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The TGF- β family of signaling proteins is widely represented throughout the animal kingdom and consists of more than 40 known members including TGF- β isoforms, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activins and inhibins. Members of this family are key modulators of cell proliferation, differentiation, matrix synthesis and apoptosis. They play major roles during prenatal development and postnatal growth, remodeling, and maintenance of a variety of tissues and organs. In accordance with their role in regulating vital biological processes, the amino-acid sequences of homologous TGF-β proteins from different species are highly conserved. The characteristic features common to all members of this family are: (I) the functional ligand is a disulfide-linked homo- or heterodimer, consisting of two 12-15 kDa polypeptide chains, called monomers, (II) each monomer is expressed as the C-terminal part of a precursor propeptide, which also contains a short N-terminal signal sequence for secretion, and a long propeptide of 200-300 amino acid residue, and (III) the primary structure of the monomer contains a highly conserved seven-cysteine domain in the C-terminal region. One of these cysteine residues is used for interchain disulfide bridging, and the others are involved in an intramolecular ring formation, known as the cystine knot configuration. The cysteine knot is a folding motif





Figure 1: Schematic illustration of the TGF-β family cysteine knot configuration

- A The structure of the TGF- β family cysteine knot monomer consists of two finger-like anti-parallel beta-strand projections (gold), and an α -helical heel region (green). Two disulfide bridges complete a ring of covalent bonds that is penetrated by a third disulfide bond to form the knot.
- ${f B}$ The characteristic seven-cysteine spacing motief in the TGF-eta cysteine-knot domain. Cysteine 4 (C4) is utilized for inter-chain disulfide bond formation.

that forces exposure of hydrophobic residues to the aqueous surrounding, and prevents the molecule from assuming a globular protein structure (Figure 1). Instead, it drives the molecules to undergo dimerization, resulting in highly stable dimeric proteins with a butterfly-shape structure. The cysteine knot structural motif is not unique to TGF- β proteins and is found in other cytokine families, such as NGF, PDGF, glycoprotein hormone (GPH) and IL-17, which together constitute a large superfamily of cysteine knot proteins.

Signaling by members of the TGF-β family involves cooperative binding of the ligand to both type I and type II transmembrane receptor components, which induces assembly of an active serine/threonine kinase receptor complex. This receptor complex initiates a signal transduction pathway by phosphorylating cytoplasmic Smad proteins, which then translocate to the nucleus and act to suppress or activate transcription of target genes. It should be noted, however, that ligand accessibility to TGF-β receptors is so highly controlled by a host of ligand- and/or receptor-binding proteins that the ligand-receptor interaction may be viewed as a downstream stage, rather than the start of the signaling pathway. BAMBI (BMP and activin membrane-bound protein) constitutes an example for a transmembrane protein which modulates TGF-β signaling by acting as a pseudoreceptor for BMP/activin type II receptors. This membrane-anchored protein forms stable but signalingincompetent complexes with type II receptors, thereby inhibiting BMP/activin signaling (1). A more widespread type of regulation involves soluble proteins that associate with TGF-β ligands to form latent complexes. In addition to providing extracellular storage sites for TGF-β ligands, these binding proteins play central roles in controlling the local concentration of TGF-β activity. Impairment in proper TGF-β activity has been associated with a variety of clinical indications, including tumor cell growth, fibrosis, skeletal defects, and autoimmune disease. Therefore, understanding the regulatory mechanisms that control TGF- β signaling, as well as the identification and characterization of their molecular components have become topics of intense investigation. Here we highlight the roles played by secretory proteins in regulating the bioactivity of TGF- β family members.

The Propeptide or Latency-Associated Protein (LAP)

After dimerization of the TGF- β precursor, the covalent bonds between the propeptide and the mature ligand are cleaved by a furin-type protease. However, the resulting two dimeric polypeptides remain associated through non-covalent interactions, and are secreted as a latent complex. The propeptide portion of this complex, also called latency-associated protein or LAP, renders the mature ligand biologically inactive by preventing accessibility to membrane receptors. For some members of this family, the propeptides have been shown to be

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potent inhibitors of their corresponding ligands. For example, overexpression of the myostatin propeptide in mice resulted in large increases (up to 200%) in skeletal muscle mass, similar to those observed in myostatin knockout mice (2). Depending upon the dissociation constant (Kd) of the latent complex, LAP may become the predominant extracellular companion of the ligand, as in the case of TGF- β 1, or allow the ligand to interact with other high-affinity binding proteins, as in the case of activin, which detaches from its LAP and associates with proteins containing follistatin (FS)-domains. Alternatively, the complexed LAP may covalently bind via disulfide bonds to large secretory glycoproteins known as latent TGF-β binding proteins (LTBPs). The physiological role of LTBPs is not fully known, but they may facilitate the secretion, storage, or activation of ligand-LAP complexes. The controlled-release of biologically active TGF-β ligands from latent complexes appears to involve proteolytic processing of LAP and/ or induction of conformational modification by proteins such as thrombospondin-1 (TSP-1) (3).

Natural TGF- β Antagonists

Once liberated from latent complexes, ligands of the TGF- β family may be intercepted by a host of diffusible proteins which bind the ligands with varying degrees of affinity and inhibit their access to signaling receptors. The expression of some of these natural antagonists is positively regulated by their TGF-β binding partners. Reverse diffusion of the antagonists, i.e., from target cells towards localized sources of competent ligands, creates signaling gradients in the extracellular milieu (4). The interplay between ligands of the TGF- β family and their natural antagonists has major biological significance during developmental processes, in which the response of cells can vary considerably depending upon the local concentration of the signaling molecule. The growing list of TGF-β natural antagonists includes noggin, chordin, chordin-like proteins, follistatin (FS), FS-related proteins, sclerostin, and the DAN/cerberus family of proteins (Table 1). A novel member in this structurally-diverse group of proteins is GDF-associated

serum protein-1 (GASP-1). GASP-1 has been identified in normal mouse and human serum as a myostatinassociated protein. The primary structure of GASP-1 reveals multiple putative protease inhibitory domains, and a cysteine-rich FS-like domain (5). Most TGF- β natural antagonists are capable of interacting with more than one ligand, but display discriminating preferences for different ligands. For example, follistatin, which binds activin in a nearly irreversible manner, displays high-affinity binding for BMP-7, and a lower affinity for BMP-2 and -4. Noggin, on the other hand, displays high-affinity binding for BMP-2 and -4, and a lower affinity for BMP-7.

References

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Table 1: Antagonists of TGF- β Ligands

Natural TGF- β Antagonists	Structural Features Contained in the Antagonist Polypeptide (m.w.)	Known TGF-β Binding Partners
Noggin	unique Noggin cysteine-knot domain (26 kDa)	BMP-2,-4,-5,-6,-7,-13/GDF-6,-14/GDF-5
Chordin	4 CR/VWC (Chordin) domains, 3 SOG repeats (102 kDa)	BMP-2,-4,-7
Chordin-like/Neuralin/Ventroptin	3 Chordin domains (51 kDa)	BMP-4,-5,-6
Follistatin	3 cysteine-rich Follistain (FS) and 3 kazal domains (38 kDa)	activin, BMP-2,-4,-6,-7, myostatin/GDF-8, GDF-11, TGF-β-1
Follistatin-like related gene (FLRG)	2 FS and 2 kazal domains (28 kDa)	activin, myostatin/GDF-8, GDF-11, TGF-β-1
GASP-1	1 Wap, 1 FS, 1 kazal, 1 IG-like, 2 kunitz, 1 netrin domains (63 kDa)	myostatin/GDF-8,GDF-11
Follistatin-related protein (FSRP)	1 FS, 1 CR/VWRC, 2 EF-hand domains (35 kDa)	activin, BMP-2,-6,-7,
DAN	unique DAN cysteine-knot domain (19 kDa)	BMP-2,-4,-7, -14/GDF-5
Cerberus	DAN-like cysteine-knot domain (30 kDa)	BMP-2,-4,-7, activin, nodal
Gremlin	DAN-like cysteine-knot domain (21 kDa)	BMP-2,-4,-7
Sclerostin/SOST	unique Sclerostin cysteine-knot domain (24 kDa)	BMP-5, -6
Decorin	multiple leucine-rich repeats (40 kDa)	TGF-β-1, -2
alpha-2 macroglobulin	multiple proteinase inhibitor domains (163 kDa)	TGF-β-1, -2, activin, inhibin