The Thrombospondins

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Thrombospondins are evolutionarily conserved, calcium-binding glycoproteins that undergo transient or longer-term interactions with other extracellular matrix components. They share properties with other matrix molecules, cytokines, adaptor proteins, and chaperones, modulate the organization of collagen fibrils, and bind and localize an array of growth factors or proteases. At cell surfaces, interactions with an array of receptors activate cell-dependent signaling and phenotypic outcomes. Through these dynamic, pleiotropic, and context-dependent pathways, mammalian thrombospondins contribute to wound healing and angiogenesis, vessel wall biology, connective tissue organization, and synaptogenesis. We overview the domain organization and structure of thrombospondins, key features of their evolution, and their cell biology. We discuss their roles in vivo, associations with human disease, and ongoing translational applications. In many respects, we are only beginning to appreciate the important roles of these proteins in physiology and pathology.

Thrombospondins (TSPs) comprise a conserved family of extracellular, oligomeric, multidomain, calcium-binding glycoproteins. In general, basal metazoa and protostomes encode a single TSP in their genomes and deuterostomes have multiple TSP genes. The TSPs of mammals have many complex tissue-specific roles, including activities in wound healing and angiogenesis, vessel wall biology, connective tissue organization, and synaptogenesis. These activities derive mechanistically from interactions with cell surfaces, growth factors, cytokines, or components of the extracellular matrix (ECM) that collectively regulate many aspects of cell phenotype. Emerging evidence on the functions of TSPs in invertebrates suggests that ancient functions include bridging activities in cell–cell and cell–ECM interactions. Knowledge of TSP domain structures provides a rational basis for understanding their roles in vivo and associations with human disease and is assisting ongoing translational applications.

DOMAIN ARCHITECTURE AND DOMAIN STRUCTURES

The domain architectures of representative TSP polypeptides are shown in Figure 1A. The invariant carboxy-terminal regions comprise a series of EGF-like domains, thirteen calcium-binding type 3 repeats, and a carboxy-terminal domain structurally homologous to the L-type lectin

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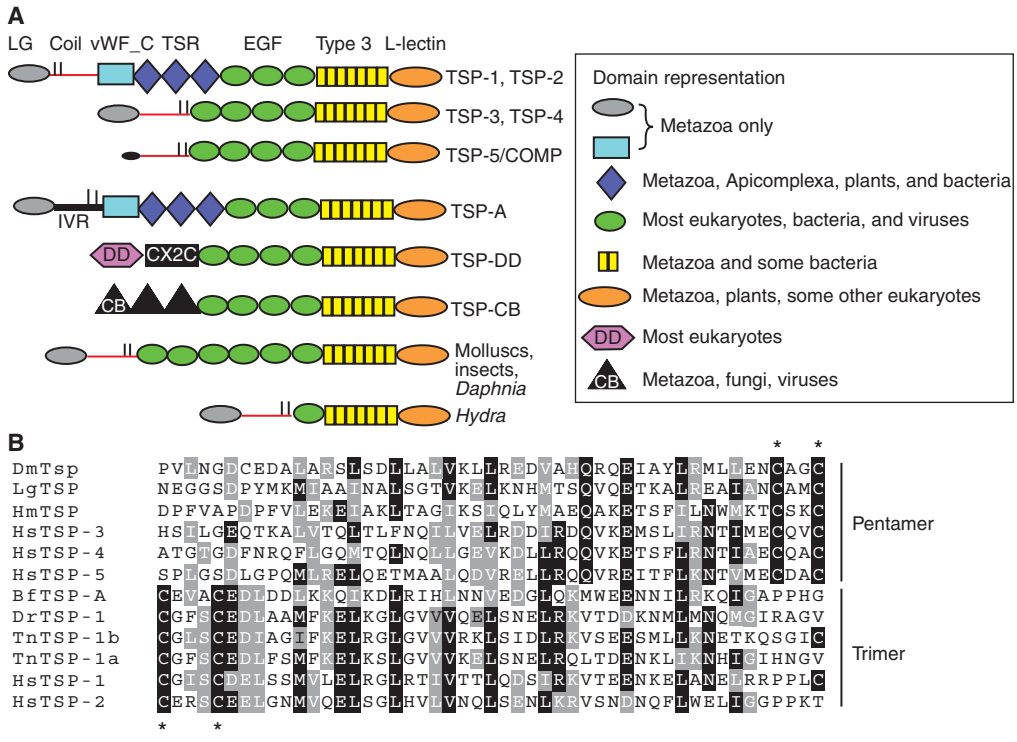

Figure 1. Domain architectures of thrombospondins. (*A*) Schematic diagram of the domain architectures of thrombospondin family members. Key: LG = laminin G-like amino-terminal domain; vWF_C = von Willebrand type C domain; TSR = thrombospondin type 1 domains; EGF = epidermal growth factor-like domains; Type 3 = thrombospondin type 3 repeats; L-lectin = L-type lectin-like domain; DD = discoidin domain; IVR = intervening region; CX2C = Cys-X2-Cys domain; CB = chitin-binding type 2 domain. Horizontal red lines indicate coiled-coil domains. Vertical black lines indicate position of cysteine residues that form intersubunit disulfide bonds. (*B*) Examples of the coiled-coil oligomerization domain from representative trimeric and pentameric thrombospondins. Asterisks indicate cysteines that form intersubunit disulfide bonds.

domain. This domain organization is the hallmark of a TSP and has also been termed the "signature" domain (Adams and Lawler 1993; Carlson et al. 2005). The amino-terminal halves of TSPs are much more varied in domain composition, with the laminin-G like (LG) amino-terminal domain (NTD) being the most widely conserved domain. The discoidin domain or Type 2 chitin-binding domains are present in some TSPs of invertebrates (Fig. 1A). A highly prevalent, although not invariant, feature of TSPs is an α-helical coiled-coil domain located adjacent to the NTD (red line in Fig. 1A) that mediates cotranslational oligomerization via formation of a left-handed super-helix. Vertebrate TSPs assemble either as trimers (subgroup A, comprising TSP-1 and TSP-2) or pentamers (subgroup B, comprising TSP-3, TSP-4, and TSP-5/COMP; TSP-5 is also known as cartilage oligomeric matrix protein [COMP]) (Lawler et al. 1985, 1995; Sottile et al. 1991; Mörgelin et al. 1992; Qabar et al. 1995). Residues important for pentamerization have been identified by mutational studies of the COMP/TSP-5 coiled-coil (Gunasekar et al. 2009). Oligomerization of TSPs is stabilized by intersubunit disulfide bonds formed between cystine residues adjacent to the amino-terminal end of the heptad repeats in trimeric TSPs or the carboxy-terminal end in pentameric TSPs (Fig. 1B) (Prochownik et al. 1989; Sottile et al. 1991; Qabar et al. 1995). Monomeric, dimeric, and pentameric TSPs exist in

invertebrates (see also section on Evolution of Thrombospondins).

Structures for the major domains of TSPs have been solved by X-ray crystallography. The NTD of TSPs 1–4 folds as a Laminin-G domain (Tan et al. 2006). The structure of the vWF_C domain of TSPs has not been determined but is predicted to conform to the vWF_C domain of collagen IIA (Fig. 2) ([Protein Data Bank—PDB 1U5M] O'Leary et al. 2004). Each thrombospondin type 1 domain (TSR) corresponds to a novel fold composed of three β-strands with alternating orientation, stabilized by three disulfide bonds and cation-π bonds between highly conserved tryptophan residues in the first strand and two arginine residues in the second strand (Tan et al. 2002). This folding pattern brings together sequences from the first and second strands to form a positively charged groove on one surface of the TSR that is thought to represent the binding site for the CD36 receptor (see section Major Binding Partners). Around 90 proteins containing TSR domains are encoded in the human genome. Of those that have been characterized functionally, many are involved in cell–cell and cell–ECM interactions and cell migration (Adams and Tucker 2000). Proteins involved in axon guidance include F-spondin, SCO-spondin, and UNC-5; others include complement factors, proteases, and protease inhibitors (Tucker 2004).

In TSP-1 and -2, the three TSRs are followed by three epidermal growth factor-like (EGF) domains. TSP-3, -4, and -5/COMP and many TSPs of invertebrates contain larger numbers of EGF-like domains contiguous with the coiled-coil domain (Fig. 1A). Crystal structures have been solved for different portions of the carboxy-terminal regions of TSP-1 ([PDB 1UX6] Kvansakul et al. 2004), TSP-2 ([PDB 1YO8] Carlson et al. 2005) and TSP-5/COMP (Fig. 2) ([PDB 3FBY] Tan et al. 2009). Multiple intramolecular interactions between the EGF-like domains, the type 3 repeats, and the L-type lectin-like domain support the concept that this entire region folds and functions as a single structural unit. The carboxy-terminal region of TSP-2 has been divided into subregions described as a stalk (EGF-like domains 2 and 3), a clasp (EGF-like domain 3), a wire

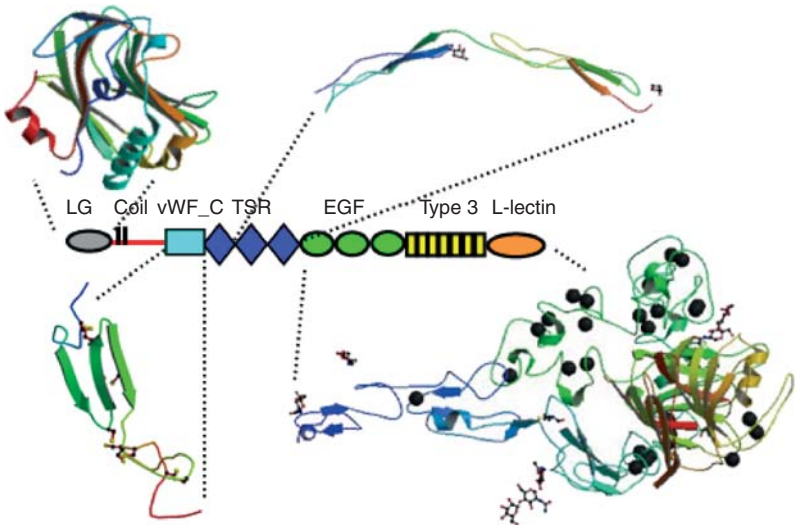

Figure 2. Structures of the domains of subgroup A thrombospondins. The crystal structures of LG (PDB 2ERF) and the second and third TSRs (PDB 1LSL) of TSP-1, and the carboxy-terminal region/signature domain of TSP-2 (PDB 1YO8) are shown. The vWF_C domain of TSP-1 is modeled on the solution structure of the vWF_C domain of collagen IIA (PDB 1U5M). Each domain is shown in a color gradient from blue at the amino terminus to red at the carboxyl terminus. The black spheres represent calcium ions. Note that the domains are not shown at the same scale.

(the type 3 repeats), and the L-type lectin domain (Carlson et al. 2005). The thirteen type 3 repeats form an unusual protein structure in which a series of 26 calcium binding sites (DxDxD/N) are stabilized by disulfide bonds between adjacent repeats, calcium, and interactions with the L-type lectin domain (Kvansakul et al. 2004; Carlson et al. 2005). Removal of calcium leads to disassociation of the type 3 repeats from the L-type lectin domain (Annis et al. 2007). Two classes of type 3 repeat motif, [N] and [C], can be distinguished by their sequence length, the way in which the calcium ions are bound, and their interactions with water molecules (Kvansakul et al. 2004; Carlson et al. 2005; Tan et al. 2009). The importance of the type 3 repeats for the correct folding of the entire carboxy-terminal region is emphasized by the fact most point or single amino acid deletion mutations of human TSP-5/COMP that lead to pseudoachondroplasia (PSACH) or multiple epiphyseal dysplasia (EDM) occur in this region and disrupt protein conformation and calcium binding (see section *TSP-5/COMP and PSACH*). Coding polymorphisms in the carboxy-terminal regions of TSP-1 or TSP-4, or COMP-equivalent mutations in TSP-2 also affect calcium-binding and protein conformation (Stenina et al. 2003; Carlson et al. 2008a,b) (see section *TSP Single Nucleotide Polymorphisms and Disease*).

The carboxy-terminal L-type lectin-like domain contains 15 β-strands in two curved antiparallel β-sheets and also binds calcium ions (Kvansakul et al. 2004). All TSPs contain the sequence DDDYAGF in the loop between the β5 and β6 strands and two calcium ions are coordinated by the DDD motif. A third calcium-binding site is in close proximity to the DDDYAGF sequence and, in TSP-1, D956 and D975 coordinates a fourth calcium ion (Kvansakul et al. 2004; Tan et al. 2009).

EVOLUTION OF THROMBOSPONDINS

TSPs are exclusive to the metazoa. However, most of their component domains have pre-metazoan origins (Fig. 1). TSP pentamers apparently arose very early in the metazoa and have been highly conserved. Most protostomes and inferred basal metazoa encode a single TSP with the general domain organization of subgroup B TSPs and with a pentamerizing coiled-coil (Figs. 1 and 3). It appears that gene

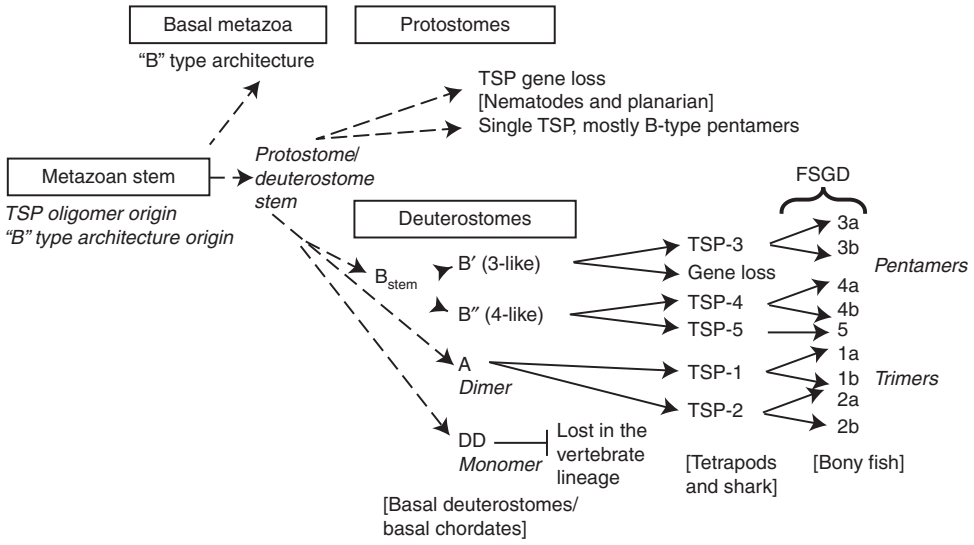

Figure 3. Model for the evolution of thrombospondins within the metazoa. FSGD = fish-specific genome duplication. (Diagram is a development of a figure originally published in Bentley and Adams [2010]. It is reprinted, with permission, from Oxford University Press © 2010.)

duplication and domain-shuffling events took place on the deuterostome stem lineage because all modern deuterostomes, urochordates, and cephalochordates encode three to four TSPs per genome. These include two novel forms: the TSP-A domain architecture and TSP-DD, a monomeric form with an amino-terminal discoidin-like domain that was lost from the vertebrate lineage (Figs. 1 and 3). Interestingly, TSP-As of *Ciona*, sea urchin, and acorn worm have all the major domains of TSP subgroup A yet do not contain a coiled-coil (Fig. 1A). The simplest explanation is that the trimerizing coiled-coils of TSP-1 and TSP-2 evolved separately from the pentamerizing coiled-coils (Bentley and Adams 2010).

TSP evolution in vertebrates involved further gene duplications, likely resulting from the genome-wide duplications that occurred early in the vertebrate lineage, plus subsequent gene losses resulting in a total of five TSP genes in modern tetrapods (Fig. 3 and Table 1). A third genome duplication took place in the ray-finned fish lineage resulting in additional paralogs (Table 1) (McKenzie et al. 2006; Wu et al. 2009). Across both bony fish and tetrapods, orthologous TSP genes display conservation of synteny (Table 1) and *Thbs3*, *Thbs4*, and *Thbs5/COMP* are located in paralogous genomic regions, indicating their evolution as duplicated genes within the vertebrate lineage (Fig. 3). Interestingly, *Thbs5/COMP* of bony fish encodes a protein that is most closely related in sequence to tetrapod TSP-4 (even though the *Thbs5/COMP* gene product of both bony fish and tetrapods lacks an LG-NTD) (McKenzie et al. 2006). These data support the model that duplication of a *Thbs4-like* gene provided the origin for *Thbs4* and *Thbs5/COMP* (Fig. 3). This model implies that TSP-5/COMP protein sequence has diverged faster in tetrapods than in bony fish, and thus might be evolving distinct functions in tetrapods.

CELL BIOLOGY OF THROMBOSPONDINS

Expression and Synthesis of TSPs

Data on the tissue expression profiles of TSPs have been collected from adult human or mouse tissues or mouse or chicken embryos; studies of other organisms are more fragmentary (Table 2). TSP-1 and TSP-5/COMP mRNA and protein are regulated by many environmental cues or pathological agents (Table 3). The synthesis of TSP polypeptides involves signal-mediated cotranslational transfer into the lumen of the endoplasmic reticulum (ER). Oligomerization of TSP-1 into trimers occurs through noncovalent association of the coiled-coil domains (Vischer et al. 1985; Prabakaran et al. 1996). Although TSPs are predominantly homooligomers, natural heteropentamers of TSP-4 and TSP-5/COMP subunits occur in tendon (Hecht et al. 1998; Södersten et al. 2006).

Quality control of TSP-1 polypeptide folding is mediated by ER chaperones (Kuznetsov et al. 1997). BiP, calreticulin, protein disulfide isomerase, ERp72, and grp94 are coretained

Table 1. Chromosomal locations of thrombospondin genes in representative vertebrates

Thbs gene	Human	Mouse	Chicken	Pufferfish *T. nigroviridis*	Zebrafish *D. rerio*
Thbs1	15q15	2 band F	5	1a:14, 1b:10	20
Thbs2	6q27	17 band A3	3	17	2a:13, 2b:12
Thbs3	1q21	3 band E3	Unmapped	Not in genome	3a:16, 3b:19
Thbs4	5q23	13-52	Z	4a:12, 4b:4	4a[a]:5, 4b:21
Thbs5/COMP	19p13.1	8-22	28	1	11[b]

The locations of *Thbs* genes show conservation of synteny across the species (McKenzie et al. 2006).

[a]*D. rerio* TSP-4 paralogs were unmapped at time of publication of McKenzie et al. (2006), and in this paper NP_775333 was designated as *D. rerio* TSP-4a. However, NP_001107896, encoded on *Danio* chromosome 5, is now known to be adjacent *metaxin-3,* i.e., to have conservation of synteny with human *THBS4*. This gene is now designated *D. rerio thbs4a*.

[b]Location according to Zv8 genome assembly.

Table 2. Tissue expression patterns of thrombospondins in vertebrates and invertebrates

TSP	Tissue sites of expression	References
Mammalian		
TSP-1	Mouse embryo: */^widespread, most prominent in heart, lung, intestinal epithelium, skeletal muscle, CNS Adult mouse or human: ^more restricted, platelet α-granules, activated endothelium, ovary, cornea, lens; in healing wounds of skin, skeletal muscle, or spinal cord; neointima, atherosclerotic plaques	Murphy-Ullrich and Mosher 1985; Raugi et al. 1987, 1990; O'Shea and Dixit 1988; Watkins et al. 1990; Corless et al. 1992; Iruela-Arispe et al. 1993; Hoffman et al. 1994; Hiscott et al. 1996, 1997; Moller et al. 1996; Roth et al. 1998; Greenaway et al. 2005
TSP-2	Mouse embryo: ^cartilage growth zone, ^skeletal muscle, ^bone, *kidney, */^adrenal gland, ^skin, *brain, *lung, *heart Adult mouse: ^Adrenal cortex, ^bone marrow stromal cells. Adult human: *brain	Laherty et al. 1992; Iruela-Arispe et al. 1993; Kyriakides et al. 1998; Tooney et al. 1998; Adolph 1999; Caceres et al. 2007
TSP-3	Mouse embryo: */^CNS, *Meckle's cartilage, *spinal cord, *lung, *bone, *skeletal muscle, *diaphragm, *intestine Adult mouse or human: *Kidney, *muscle, intestine, *lung, *heart, *tail, *skin, *bone, *skeletal muscle	Vos et al. 1992; Iruela-Arispe et al. 1993; Lawler et al. 1993; Qabar et al. 1994
TSP-4	Adult mouse, rat, or human: *Heart, */^skeletal muscle, *diaphragm, ^tendon, ^neuromuscular junction, ^cerebellum, */^hippocampus, *cerebral cortex, ^retina, ^blood vessels Adult mouse: ^adventita of arteries, atherosclerotic lesions	Lawler et al. 1993; Arber and Caroni 1995; Hauser et al. 1995; Chen et al. 2000; Stenina et al. 2003; Caceres et al. 2007; Frovola et al. 2010
TSP-5/ COMP	Mouse embryo: *skeletal muscle and all cartilaginous tissue Adult mouse or human: ^articular cartilage, ^synovium, ^tendon, ^skeletal muscle, ^testis, ^arteries, ^eye, ^heart	Franzen et al. 1987; DiCesare et al. 1994a,b, 1997; Fang et al. 2000; Kipnes et al. 2000; Riessen et al. 2001; Wilson et al. 2010
Avian TSP-1 TSP-2 TSP-3 TSP-4	*Gallus gallus* embryo: *CNS and floorplate, cartilage *Cartilage growth zone, tendon *CNS, spinal cord, lung, bone *Cornea, early osteogenic tissue	Tucker 1993; Tucker et al. 1995, 1997
Amphibian TSP-1 TSP-3 TSP-4	*Xenopus laevis* embryos: *Fertilized eggs, in embryo after gastrulation. In tadpole floor plate of neural tube, epidermis, somites, notochord, and alternating rhombomeres *In embryo after gastrulation. In tadpole notochord, floor plate, sensorial layer of the epidermis, and sensory epithelia *In embryo after gastrulation. In tadpole somitic mesoderm and skeletal muscle	Lawler et al. 1993; Urry et al. 1998

Continued

Table 2. *Continued*

TSP	Tissue sites of expression	References
Bony Fish		
TSP-1	*Danio rerio* early embryos	Wu et al. 2009; Zhou et al. 2009
TSP-1a TSP-1b	*Oreochromis niloticus* and *Oryzias latipes*: *Adult ovary with dynamic expression during the spawning cycle, granulosa cells, skeletal system, brain, intestine, heart, spleen	
	Oreochromis niloticus and *Oryzias latipes*: *Gonads, theca cells of adult ovary, skeletal system, heart, spleen	Wu et al. 2009
TSP-2	*Solea senegalensis*: *in ovary, 2x up-regulated in atretic ovary relative to vitellogenic/mature ovary	Tingaud-Sequeira et al. 2009, EST accessions FF284909, FF284981
TSP-5/ COMP	*Solea senegalensis*: *in ovary, 2x up-regulated on vitellogenesis	
Invertebrate	Embryo: *trunk mesoderm, wing imaginal disc,	Adams et al. 2003; Chanana et al. 2007;
Drosophila TSP	tendon cells of pharyngeal muscles, */^myotendinous junction	Subramanian et al. 2007
Prawn TSP-CB	*Marsupenaeus japonicus*: */^in cortical rods of vitellogenic and mature oocytes	Yamano et al. 2004
	Fennerpenaeus chinensis: *in hemocytes, heart, intestine, stomach and ovary, induced in hepatopancreas on microbial challenge	Sun et al. 2006
	Penaeus monodon: *in ovary	Preechaphol et al. 2007
	Penaeus monodon: *up-regulated in lymphoid organ on *Vibrio harveyi* infection	Pongsomboon et al. 2008

*Transcript, ^protein.

with TSP-5/COMP in the Golgi of chondrocytes from PSACH patients (see section ROLES OF TSPS IN VIVO), suggesting that these chaperones also participate in its normal quality control (Hecht et al. 2001; Vranka et al. 2001). Trafficking of TSPs from ER to Golgi appears to be by COPII vesicles (Veliceasa et al. 2007). Sec23a-positive vesicles are implicated in trafficking wild-type TSP-5/COMP to the Golgi in chondrocytes (Fig. 4) (Saito et al. 2009).

While transiting the secretory pathway, TSP-1 becomes modified by N- and O-linked sugars (Furukawa et al. 1989; Nishimura et al. 1992). The TSRs undergo C-mannosylation of tryptophan residues within the WXXW motifs (Hofsteenge et al. 2001) and are also modified by the unusual disaccaride Glc-Fuc-O-Ser/Thr through the actions of protein O-fucosyl transferase 2 (POFUT2) and β1,3-glucosyltransferase, (β3GLT) (Kozma et al. 2006; Luo et al. 2006; Sato et al. 2006). The biological roles of these modifications of TSRs remain unclear; however, mutations of β3GLT cause a genetic disorder, Peters Plus syndrome (Hess et al. 2008).

Generally, TSPs are secreted from cells by constitutive pathways; an exception is the release of TSP-1 from stored platelet α-granules that are discharged on platelet activation (Blair and Flaumenhaft 2009). In apico-basally polarized cells, TSP-1 secretion is targeted to the basolateral membranes (Prabakaran et al. 1993, 1999; Gath et al. 1997).

Degradation of TSPs

Extracellular. After secretion, TSPs can be incorporated into extracellular matrices in cell culture and in vivo (Raugi et al. 1982; Jaffe et al. 1983; Vischer et al. 1985; DiCesare et al. 1994a; Schlötzer-Schrehardt et al. 2007; Adams

Table 3. Factors that regulate TSP-1, TSP-4, and TSP-5

Factor	Regulation /cell type	Reference
Amino acids	Increased TSP-1 in glomerular mesangial cells	Meek et al. 2003
Angiotensin II	Increased TSP-1 synthesis in vascular smooth muscle cells	Scott-Burden et al. 1990
Cardiac overload	Increased TSP-1 and TSP-4 transcripts in left ventricle	Mustonen et al. 2008
Extracellular ATP	Increased TSP-1 production by dendritic cells	Marteau et al. 2005
Glucose	Increased TSP-1 synthesis by mesangial cells and vascular smooth muscle cells	Tada and Isogai 1998; Wang et al. 2004; Raman et al. 2007
Heat shock	TSP-1 in endothelial cells	Ketis et al. 1988
Herpes simplex virus type 1	Suppression of TSP-1 transcript in endothelial cells	Ziaie et al. 1986
Hypoxia	Increased TSP-1 transcript and protein in endothelial cells	Phelan et al. 1998
Id-1	Transcriptional repression of TSP-1; modulates angiogenesis	Volpert et al. 2002a
KSHV	Transcriptional silencing of TSP-1 by viral microRNAs	Samols et al. 2007
Mechanical cyclic compression	Increased TSP-5/COMP transcript in articular cartilage explants	Giannoni et al. 2003
Nedd4 ubiquitin ligase	Suppression of TSP-1 transcript in MEFs and heart	Fouladkou et al. 2010
PDGF, HS-GAGs	TSP-1 synthesis in vascular smooth muscle cells	Majack et al. 1985
TGFβ1	Increased TSP-5/COMP synthesis by chondrocytes and synovial fibroblasts	Recklies et al. 1998
Ultraviolet B	Decreased TSP-1 transcript in keratinocytes; increased TSP-1 transcript in dendritic cells	Howell et al. 2004; de la Fuente et al. 2009

et al. 2008). Alternatively, proteolytic fragments can be generated that either have a specific extracellular activity (Lee et al. 2006), or are internalized for full degradation (see below) (Fig. 4). Extracellular proteolysis of TSP-1 by thrombin or plasmin occurs during fibrinolysis and fibrin clot resolution (Lawler and Slayter 1981; Dixit et al. 1984; Bale and Mosher 1986) or during inflammation by elastase (Raugi et al. 1984; Hogg et al. 1993). Cleavage of TSP-1 by ADAMTS-1 releases antiangiogenic fragments (Lee et al. 2006). TSP-5/COMP is a substrate for MMP-19/-20 and ADAMTS-4/-7/-12 (Stracke et al. 2000; Dickinson et al. 2003; Liu et al. 2006a,b) and increased COMP fragments in synovial fluid are correlated with joint damage in rheumatoid arthritis and osteoarthritis (Neidhart et al. 1997).

Intracellular. TSP-1 and TSP-2 are endocytosed for intracellular degradation within lysosomes: the rate depends on the cell type and the expression of cell-surface glycosaminoglycans (McKeown-Longo et al. 1984; Murphy-Ullrich and Mosher 1987a,b; Murphy-Ullrich et al. 1988; Chen et al. 1996a). For TSP-1, endocytosis is mediated by binding of its LG-NTD to a ternary cell-surface complex of LDLR-related protein 1 (LRP1), extracellular calreticulin, and heparan sulphate proteoglycans (Fig. 4) (Godyna et al. 1995; Mikhailenko et al. 1995, 1997; Chen et al. 1996b; Orr et al. 2003; Wang et al. 2004a).

Major Binding Partners

TSPs have many binding partners; the best-validated are listed in Table 4. Integrin-binding by TSPs is important for their activities in cell attachment, spreading, and migration. The best-characterized interaction is that of the

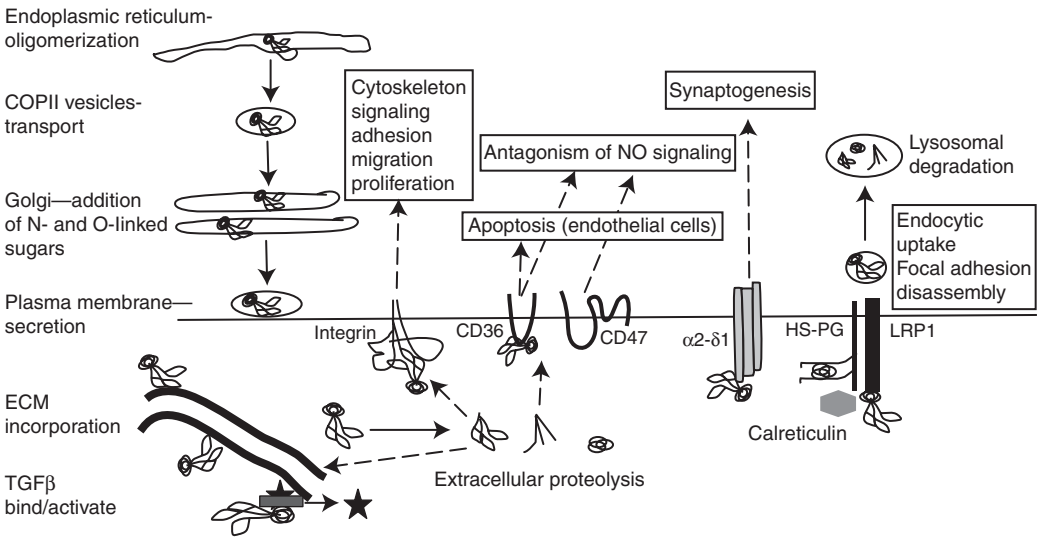

Figure 4. Overview of cellular pathways and activities of mammalian TSP-1 (not to scale).

single RGD motif of TSP-1 with integrin αvβ3 and, to a lesser extent, with αIIbβ3 (Table 4) (Lawler et al. 1988; Lawler and Hynes 1989). The availability of this RGD motif for integrin-binding is promoted by incomplete calcium ion loading or reduction of disulfide bonds within the type 3 repeats (Sun et al. 1992; Kvansakul et al. 2004). Many cells undergo RGD-independent attachment to calcium-replete TSP-1 or TSP-2 (reviewed in Adams 2004). Many TSPs contain RGD and KGD potential integrin-binding motifs at other locations in the type 3 repeats. Few of these have been tested functionally, however, the RGD motif of TSP-5/COMP is implicated in binding α5β1 and, under reducing conditions, αvβ3 (Chen et al. 2005). The KGD motif of *Drosophila* TSP is needed for αPS2 integrin-dependent cell adhesion in vitro (Subramanian et al. 2007). TSP-1 and TSP-2 also bind several non-RGD-dependent integrins including α4β1 (Table 4). Binding sites for integrins α3β1 and α6β1 have been mapped to the LG-NTD, yet the physiological significance of these remains uncertain because the identified motifs are not fully surface-exposed in the crystal structure (Krutzsch et al. 1999; Calzada et al. 2003; Tan et al. 2006). However, α3β1 binding may be favored in calcium-depleted TSP-1 (Rodrigues et al. 2001). β1 integrins are also implicated in interactions with the TSRs and EGF-like domains (Calzada et al. 2004b).

ECM incorporation is a conserved property of TSPs and, through their multivalent structures, TSPs likely function as molecular bridges to facilitate ECM organization. Incorporation of TSP-1 into culture ECM depends on the carboxy-terminal region in trimeric form. This activity is partially inhibited by mutation of the three highly conserved aspartic acid residues that coordinate two calcium ions in the L-lectin domain (see section DOMAIN ARCHITECTURE AND DOMAIN STRUCTURES) (Adams et al. 2008). The DDD motif is also part of a motif in TSP-5/COMP reported to bind collagen IX (Table 4) (Holden et al. 2001). In vitro, TSP-5/COMP acts as a catalyst for collagen fibrillogenesis (Halász et al. 2007; Hansen et al. 2011). Other important interactions are with glycosaminoglycans. Cocrystals of the TSP-1 LG-NTD with heparin oligosaccharides revealed that R29, R42, and R77 form a positively charged patch that binds to sulfate groups on the heparins (Tan et al. 2006, 2008). Molecular docking studies indicate that longer heparins might also interact with other

Table 4. Mapped binding partners

TSP domain	Motif	Binding partner	Reference
LG-NTD	Positive patch involving R29,K32,R42,R77,K80,K81, K106[a]	HS-glycosaminoglycans	Lawler et al. 1992; Tan et al. 2006
	MKKTRG[a]	Decorin	Merle et al. 1997
	E17LTGAARKGSGRRLVKGPD[a]	Calreticulin	Murphy-Ullrich et al. 1993; Goicoechea et al. 2000
	A159ELDVP[a]	α4 Integrin	Calzada et al. 2004a
	I151DCEKMENAELDVP[a]	Fibrinogen	Voland et al. 2000
Type 1 repeats	WSXWS[e]	HS-glycosaminoglycans	Guo et al. 1992
	CSVTCG[e]	CD36	Asch et al. 1992
	W420SHWSPW[c]	TGF-β binding	Schulz-Cherry et al. 1995
	K412RFK[b]	TGF-β activation[b]	Ribeiro et al. 1999
Type 3 repeats	RGD[a,d]	β1 Integrin, β3 integrin	Lawler et al. 1988; Lawler and Hynes 1989; Chen et al. 2005;
	KGD	PS2 Integrin	Chanana et al. 2007; Subramanian et al. 2007
L-type lectin domain	GVDFEGTFHVNTVTDDD	Fibrillar collagen[d] Collagen IX[d] Matrilin-3[d]	Holden et al. 2001

Binding partners of thrombospondins. The interactions listed are those for which the binding site has been mapped within the relevant TSP domain and is surface-exposed in the domain structure, as determined by X-ray crystallography.

[a]Identified in TSP-1.

[b]Specific to TSP-1.

[c]Present in the second type 1 domain of both TSP-1 and TSP-2.

[d]Identified for TSP-5/COMP. The DDD motif is also surface-exposed in TSP-1 and TSP-2 and is conserved in most TSPs.

positively charged residues and bridge between LG-NTDs; this might contribute to the high affinity of heparin binding by TSP-1 (San Antonio et al. 1993). R29 and R42 are in a 26 aa segment absent from TSP-3 or TSP-4, thus these TSPs probably engage heparin through other positively charged residues in LG-NTD. TSP-5/COMP has no LG-NTD yet binds with high affinity to chondroitin sulfate and heparin (Chen et al. 2007); this is likely mediated by positively charged patches on the surface of the type 3 repeats and L-lectin domain (Tan et al. 2009). Interactions with other ECM ligands are, as yet, unmapped (Table 5).

Other interactions of TSPs are with growth factors and proteases. The interaction with TGFβ1 is particularly complex and is specific to TSP subgroup A members. The WSHWSPW motif located in the second TSR of TSP-1 and TSP-2 binds to VLAL motifs present in both TGFβ1 and its latency-associated peptide that together form the small latent complex (SLC) (Schultz-Cherry et al. 1995; Young and Murphy-Ullrich 2004). Binding of SLC may serve to localize inactive TGFβ1 at specific sites within ECM or in proximity to cell surfaces. In addition, TSP-1 specifically activates TGFβ1 by triggering its dissociation from SLC by an interaction of the KRFK motif (located between the first and second TSR) with a LSKL motif proximal to the amino terminus of the latency-associated peptide (Schultz-Cherry et al. 1995; Ribeiro et al. 1999). The TSRs of TSP-1 and TSP-2 also interact with matrix metalloprotease-2 and -9 (MMP-2 or MMP-9) and this inhibits MMP activity (Bein and Simon 2000). TSP-2 also modulates the extracellular levels of MMP-2 because of endocytosis of TSP-2/MMP2 complexes by LRP1 (Yang et al. 2000, 2001).

TSP-1 binds to vascular endothelial cell growth factor (VEGF), a potent proangiogenic factor that is opposed in certain physiological

Table 5. Unmapped binding partners

TSP domain	Binding partner	Reference
LG-NTD	Link domains of versican and TSG-6	Kuznetsova et al. 2006
TSR	Collagen V	
	Glycosaminoglycans (low affinity binding)	Takagi et al. 1993
	MMP-2, MMP-9	Bein and Simon 2000
Carboxy-terminal region (EGF to L-lectin domain)	von Willebrand factor multimers[b]	
	Fibrillar collagens (TSP-1, TSP-4, TSP-5)	Pimanda et al. 2004
	Laminin, fibronectin, matrilin-2 (TSP-4)	DiCesare et al. 2002
	Fibronectin (TSP-5)	Galvin et al. 1987
	Aggrecan (TSP-5)	Rosenburg et al. 1998; Narouz-Ott et al. 2000; Thur et al. 2001; Mann et al. 2004; Chen et al. 2007

Binding partners of thrombospondins—domain assigned but unmapped interactions.
HS, heparan sulphate.
[a]Identified in TSP-1.
[b]Specific to TSP-1.
[c]Present in the second type 1 domain of both TSP-1 and TSP-2.
[d]Identified for TSP-5/COMP. The DDD motif is also surface-exposed in TSP-1 and TSP-2 and is conserved in most TSPs.
[e]These motifs are present in each of the type 1 domains of TSP-1 and TSP-2.

situations or tumors by antiangiogenic activities of TSP-1 and TSP-2 (see section *Endothelial Cells and Antiangiogenesis*). In the ovary, VEGF binding to TSP-1 results in endocytosis and degradation via LRP1 (Greenaway et al. 2007). In endothelial cells, CD36 and β1 integrin associate in *cis* with VEGF receptor 2 (VEGFR2) and signaling by VEGFR2 is modulated by the level or activity of TSP-1 (Zhang et al. 2009). Also of interest is the binding of Notch3 and its ligand Jagged1 by TSP-2, which increases ligand-dependent signaling through the Notch pathway. This activity depends on Notch3 extracellular domain and the presence of LRP1 on the ligand-producing cells for the endocytic uptake of cleaved Notch3 extracellular domain (Meng et al. 2010). Complexes of TSP-1 with Notch3 and Jagged1 do not potentiate Notch signaling (Meng et al. 2009).

It is interesting that many binding activities of mammalian TSPs represent either co-evolutionary innovations in the deuterostome lineage (for example, TGFβ1 binding by the TSR, or fibrinogen binding by LG-NTD), or neo-functions of ancient molecules such as CD36 or calreticulin. In contrast, binding to glycosaminoglycans, fibrillar collagen, or RGD-dependent integrins represent widely conserved and likely ancestral activities. CD47 is encoded only in amniotes and thus cannot be an evolutionarily ancient ligand of TSP-1 (Bentley and Adams 2010). These findings help us distinguish which interactions might be most appropriate for building synthetic ECM, or as therapeutic targets distinct from ECM organization.

Functions of TSPs at Cellular Level

Fundamental properties attributed to all TSPs examined to date include interactions with ECM components and glycosaminoglycans and support of calcium-dependent cell attachment. Other activities, investigated with regard to particular TSP family members or cell types, include the induction of cell spreading with organization of actin-based protrusions, cell migration, disassembly of focal adhesions, cell-dependent stimulation or inhibition of cell proliferation or apoptosis, stimulation of synaptogenesis by neuronal cells, and antagonism of nitric oxide signaling in vascular cells (reviewed by Adams 2001, 2004; Bornstein et al. 2004; Zhang and Lawler 2007; Isenberg et al. 2009). Here, we summarize the cellular activities of TSPs that underlie their

roles in cell–cell and cell–ECM interactions. The biological significance of these activities is discussed in the section ROLES OF TSPS IN VIVO.

Endothelial Cells and Antiangiogenesis

TSP-1 and TSP-2 are specific activators of apoptosis in microvascular endothelial cells (Dawson et al. 1997, 1999). This leads to inhibition of endothelial tubule formation in vitro, and the antiangiogenic activities of TSP-1 and TSP-2 in vivo (see section ROLES OF TSPS IN VIVO). At the molecular level, the TSRs interact with the transmembrane glycoprotein CD36, likely via the positively charged groove of the TSR (Fig. 4) (Asch et al. 1992; Dawson et al. 1997; Jiminez et al. 2000, 2001; Simantov et al. 2005; Yee et al. 2009). Additional motifs within the TSRs, implicated in heparin-binding activity, also contribute to antiangiogenic activity (Iruela-Arispe et al. 1999).

CD36 is a multifunctional 88kDa glycoprotein with two small cytoplasmic domains at its amino and carboxyl termini. TSP binding involves a short region of the extracellular domain (Asch et al. 1992; Frieda et al. 1995). Inhibition of angiogenesis by TSP-1 depends on residues in the carboxy-terminal cytoplasmic domain (Primo et al. 2005). Ligation of CD36 by TSP-1 or -2 results in intracellular association of Src family kinases, fyn or yes, activation of their kinase activities, and phosphorylation of caspases and JNK leading to apoptosis (Jimenez et al. 2001). Binding of TSP-1 to CD36 also increases expression of death receptors and Fas ligand, thereby sensitizing endothelial cells to apoptosis (Volpert et al. 2002b; Ren et al. 2009). Cell cycle progression and MAP kinase signaling in microvascular endothelial cells are also limited by a nonapoptotic mechanism involving association of the carboxy-terminal region of TSP-2 and VLDL receptor (Oganesian et al. 2008). Antiangiogenesis by TSP-1 and TSP-2 has aroused great interest as a possible therapeutic strategy to block tumor angiogenesis or treat diabetic retinopathy (see sections ROLES OF TSPS IN VIVO and TRANSLATIONAL APPLICATIONS).

Smooth Muscle Cell Migration and Proliferation

TSP-1 is elevated in the neointima of injured arteries or atherosclerotic plaques (Table 2). In cell culture, TSP-1 supports smooth muscle cell (SMC) adhesion, proliferation, and migration (Majack et al. 1986; Yabkowitz et al. 1993; Patel et al. 1997). Under conditions of elevated nitric oxide (NO), the effect of TSP-1 is reversed to inhibit these cell behaviors. This is mediated by TSP-1 binding to CD36 on SMC, resulting in reduced intracellular cyclic GMP (cGMP) levels (Isenberg et al. 2006a, 2007a). Activation of SMC migration by TSP-1 is mediated by $\alpha v\beta 3$ and $\beta 1$ integrins (Lymn et al. 2002; Isenberg et al. 2005) and stimulates assembly of fascin-containing cell protrusions (Anilkumar et al. 2002).

For both smooth muscle cells and endothelial cells, TSP-1 modulates adhesion and promotes motility by antagonizing focal adhesion assembly in response to ECM components such as fibronectin. This activity depends on a motif in the LG-NTD and is mediated by cGMP- and PI 3-kinase-dependent signaling (Murphy-Ullrich et al. 1996; Greenwood et al. 2000). These signals are transduced by a complex of LRP1, cell-surface calreticulin and LG-NTD (Fig. 4) (Orr et al. 2003). Signaling from this complex via Akt also promotes cell survival (Pallero et al. 2008).

Antagonism of Nitric Oxide Signaling

Regulation of NO signaling by TSP-1 in the vasculature affects SMC, endothelial cells and platelets (reviewed by Isenberg et al. 2009). NO is an important regulator of tissue perfusion, platelet function, and vascular tone that is synthesized and released by endothelial cells and enters vascular SMC or platelets by diffusion. In all these cells, the intracellular activity of NO is to bind and activate soluble guanylate cyclase to increase cGMP; this decreases SMC contractility, reduces platelet adhesion and aggregation, and has biphasic effects on endothelial cell proliferation. TSP-1 and TSP-2 inhibit NO-dependent stimulation of proliferation (Isenberg et al. 2005). TSP-1

also counteracts SMC relaxation by NO and increases contractility and the antithrombotic activity of NO on platelets (Isenberg et al. 2006a, 2007b, 2008a). In endothelial cells, the relationship between TSP-1 and NO is complex and triphasic. Low doses of NO are proangiogenic because of suppression of TSP-1 production (Ridnour et al. 2005), and picomolar concentrations of TSP-1 inhibit NO signaling in both endothelial cells and SMC (Isenberg et al. 2005, 2006a). Antagonism of NO signaling by TSP-1 depends on CD36 and the inhibition of myristate uptake by CD36 (Isenberg et al. 2005, 2006a, 2007a). However, in CD36-null cells, NO signaling is inhibited by a mechanism dependent on the immunoglobulin superfamily member CD47 (Isenberg et al. 2006b).

Chondrocytes and Osteoblasts

All five TSPs of mammals are present in cartilage and bone where they have roles in cell–ECM interactions (reviewed by Hankenson et al. 2010). TSP-1 inhibits mineralization by osteoblastic cells or retinal pericytes (Canfield et al. 1996; Ueno et al. 2006), whereas TSP-2 promotes mineralization by preosteoblasts (Alford et al. 2010). TSP-5/COMP produced by chondrocytes is important for the organization of other matrix components; for example, *Thbs5*$^{-/-}$*: colIIX*$^{-/-}$ cultured chondrocytes incorporate less matrilin-3 into their ECM as compared to wild-type chondrocytes (Blumbach et al. 2009) (see also section *TSP-5/COMP and PSACH*).

Skeletal Muscle

Skeletal myoblasts adhere and migrate on TSP-1 or TSP-2 because of formation of fascin-based protrusions. This response depends on trimeric assembly of the carboxy-terminal region (Anilkumar et al. 2002). Whereas ligation of syndecan-1 by TSP-1 strongly activates F-actin bundling by fascin, the ligation of integrin $\alpha5\beta1$ by fibronectin promotes protein kinase C-dependent phosphorylation of fascin, thereby inhibiting its actin-bundling activity (Adams et al. 1999; Anilkumar et al. 2003). Muscle explant cultures have shown multiple roles of TSP-1 in muscle, involving modulation of collagen $\alpha1$ and $\alpha2$ secretion that impacts endothelial cell outgrowth and proliferation, and also modulation of SMC migratory capacity (Zhou et al. 2006).

Neuronal Cells

Interactions with neuronal cells are shared properties of mammalian trimeric and pentameric TSPs. Both TSP-1 and TSP-4 support neurite outgrowth (O'Shea et al. 1991; Arber and Caroni 1995); in the case of TSP-4, this may involve interplay with laminin (Dunkle et al. 2007). TSP-1 is also important for developmental neuronal cell migration in the rostral migratory stream. In these cells, TSP-1 binds ApoER2 and VLDLR to induce phosphorylation of the intracellular signaling protein, Dab1 (Blake et al. 2008).

All TSPs secreted by mammalian astrocytes promote assembly of excitatory glutamatergic synapses within the CNS. TSP-induced synapses in culture are ultrastructurally normal and presynaptically active, but lack postsynaptic activity (Christopherson et al. 2005; Eroglu et al. 2009). TSPs do not promote inhibitory GABAergic synaptogenesis (Hughes et al. 2010). Glutamatergic synapatogenic activity is mediated by interaction of the EGF-like domains of TSPs with the vWF_A domain of $\alpha2\delta$-1, a ubiquitously expressed, nonessential subunit of L-type calcium channel that is the target of the drug gabapentin (Eroglu et al. 2009). Synaptogenesis as a result of this interaction is independent of the cytoplasmic domain of $\alpha2\delta$-1; thus, it is likely that additional downstream processes are required for the necessary cytoskeletal and membrane reorganizations, the nature of which remain to be established. In hippocampal neurons, a TSP-1/neuroligin 1 interaction was implicated in promoting synaptogenesis (Xu et al. 2010). Collectively, the data suggest that synaptogenic activity of TSPs is mediated via a multiprotein complex on neuronal cell surfaces (see also article by Barros et al. 2010).

ROLES OF TSPS IN VIVO

Analyses in *Drosophila* and Mice

Drosophila

Drosophila TSP (D-TSP) is a pentameric, heparin-binding glycoprotein that incorporates into ECM in culture (Adams et al. 2003). In embryos, D-TSP expressed at segmental boundaries is under control of hedgehog signaling in tendon precursor cells, or the transcription factor *stripe* in differentiated tendon cells (Chanana et al. 2007). D-TSP colocalizes with tiggrin in the ECM at tendon/muscle cell attachment sites. In embryos lacking D-TSP, the longitudinal muscles detach from tendon cells once muscle contractions begin, resulting in lethality. Although tiggrin, PS1, and PS2 integrins are expressed normally by muscle cells in *tsp* mutant embryos, these proteins do not polarize properly at tendon/muscle cell attachment sites, suggesting that D-TSP is important for organization of the tendon ECM. D-TSP has been identified as a PS2 integrin ligand by both genetic and functional criteria (Chanana et al. 2007; Subramanian et al. 2007). This interaction is regulated by another secreted protein of tendon cells, slowdown. In vitro, slowdown undergoes KGD-modulated association with D-TSP, suggesting that it acts by steric competition (Gilsohn and Volk 2010).

Mice

Phenotypes of mice homozygous for "knockouts" of single TSP genes are summarized in Table 6. All single gene knockouts are viable, as are double *Thbs1*$^{-/-}$; *Thbs2*$^{-/-}$ mice (Agah et al. 2002) and triple *Thbs1*$^{-/-}$; *Thbs3*$^{-/-}$; *Thbs5*$^{-/-}$ mice (Posey et al. 2008). Whereas columnar stacking of chondrocytes in growth plates is mildly disrupted in *Thbs5*$^{-/-}$ mice, the triple *Thbs1*$^{-/-}$; *Thbs3*$^{-/-}$; *Thbs5*$^{-/-}$ results in a stronger phenotype (Posey et al. 2008). In relation to the action of astrocyte-secreted TSPs on synaptogenesis (see section *Neuronal Cells*), formation of synapses in the developing brains of *Thbs1*$^{-/-}$ *or Thbs2*$^{-/-}$ mice is indistinguishable from that of wild-type mice. However, *Thbs1*$^{-/-}$; *Thbs2*$^{-/-}$ mice have a 40% decrease in the number of synapses by postnatal day 8 (Christopherson et al. 2005). *Thbs1*$^{-/-}$; *Thbs2*$^{-/-}$ mice recover poorly after experimentally induced stroke, with reduced synaptic recovery and axonal sprouting, indicative of lifelong roles for TSPs in synaptic plasticity (Liauw et al. 2008).

Many other phenotypes of *Thbs1*$^{-/-}$ and *Thbs2*$^{-/-}$ null mice (Table 6) stem from the roles of TSP-1 and TSP-2 in inhibiting angiogenesis or suppressing nitric oxide signaling (see section CELL BIOLOGY OF THROMBOSPONDINS). *Thbs1*$^{-/-}$ mice have increased blood vessel density in cardiac and skeletal muscle, retina, and iris (Table 6) (Cursiefen et al. 2004). In contrast, vascular permeability response to VEGF is significantly diminished in *Thbs1*$^{-/-}$ mice (Zhang et al. 2009). One antiangiogenic mechanism of TSP-1 is by promoting clearance of VEGF (see section Major Binding Partners), and *Thbs1*$^{-/-}$ retinal endothelial cells display changes in the distribution of Src family kinases (Wang et al. 2006; Sun et al. 2009). Increased MMP activity in the pericellular space of *Thbs1*$^{-/-}$ and *Thbs2*$^{-/-}$ mice affects both cell adhesion and angiogenesis (Rodriguez-Manzaneque et al. 2001; Maclauchlan et al. 2009) In *Thbs2*$^{-/-}$ mice, increased MMP-2 activity leads to degradation of tissue transglutaminase, decreased integrin activity and weaker collagen fibrils (Agah et al. 2005).

Thbs1$^{-/-}$ and *Thbs2*$^{-/-}$ mice have opposite phenotypes in wound healing models (Table 6). Because TSP-1 is delivered to wounds by platelets at the time of injury, wounds in *Thbs1*$^{-/-}$;*Thbs2*$^{-/-}$ mice follow the delayed healing pattern of *Thbs1*$^{-/-}$ mice (Agah et al. 2002). If the survival of the tissue is limited by ischemia, as in experimental models of kidney ischemia/reperfusion injury or the cutaneous flap assay, *Thbs1*$^{-/-}$ mice recover better than wild-type controls as a result of increased tissue perfusion in the absence of NO signaling suppression (Table 6) (Thakar et al. 2005; Isenberg et al. 2007b). *Thbs1*$^{-/-}$-specific phenotypes are caused by decreased levels of activated TGFβ (Miao et al. 2001; see section CELL BIOLOGY OF THROMBOSPONDINS). TSP-1 activates TGFβ in wound healing, immune response,

Table 6. Constitutive and experimentally induced phenotypes of *Thbs* gene knockout mice

TSP	Mouse gene knockout phenotype	Reference
TSP-1	Decreased embryonic viability	Lawler et al. 1998
	Spinal lordosis	
	Pneumonia from 1 month after birth	
	Reduced active TGFβ in lung and pancreas	Crawford et al. 1998
	Decreased survival and osteosarcoma incidence in p53-null mice	Lawler et al. 2001
	Delayed healing of skin wounds	Agar et al. 2002
	Increased vascular density during retinal development	Wang et al. 2003
	Reduced plasma vWF multimer size	Pimanda et al. 2004
	Reduced inflammation and proteinurea in experimental glomerulonephritis	Hochegger et al. 2004
	Increased inflammatory response and granulation tissue in healing myocardial infarcts	Frangogiannis et al. 2005
	Reduced platelet vessel wall adherence and thrombus formation on endothelial injury	Bonnefoy et al. 2006
	Reduced smooth muscle cell activation and neointima formation after carotid artery ligation	Moura et al. 2007
	Reduced active TGFβ in glomeruli after experimental diabetic nephropathy	Daniel et al. 2007a
	Increased tissue survival after ischemic injury	Isenberg et al. 2007b
	Decreased age-linked susceptibility to ischemic injury	Isenberg et al. 2007c
	Resistance of soft tissue to radiation injury	Isenberg et al. 2008b
	Increased susceptibility and angiogenic response to experimental inflammatory bowel disease	Punekar et al. 2008
	Accelerated atherosclerotic plaque maturation in $ApoE^{-/-}$ mice	Moura et al. 2008
	Increased cardiac and skeletal muscle capillarity and exercise capacity	Malek et al. 2009
TSP-2	Fragile skin, lax tendons with enlarged collagen fibrils	Kyriakides et al. 1998
	Twofold increase in bone density	
	Cortical thickening of long bones	
	Increased vascular density	
	Prolonged bleeding time	
	Accelerated healing of skin wounds	Kyriakides et al. 1999a
	Increased vascularity of foreign body reaction	Kyriakides et al. 1999b
	Altered organization of fibrotic capsule	
	Increased proliferation of osteoblast precursor cells	Hankenson et al. 2000
	Accelerated skin carcinogenesis with increased tumor angiogenesis	Hawighorst et al. 2001
	Increased inflammation and angiogenesis in delayed-type hypersensitivity reaction	Lange-Asschenfeldt et al. 2002
	Altered bone marrow ultrastructure and megakaryocyte differentiation	Kyriakides et al. 2003
	Increased susceptibility to angiotensin II-induced fatal cardiac rupture	Schroen et al. 2004
	Increased osteoblastogenesis and decreased bone resorption after ovariectomy	Hankenson et al. 2005a
	Increased endocortical bone formation in response to mechanical load	Hankenson et al. 2006
	Increased early phase inflammatory response and MMP-2 activity in experimental glomerulonephritis	Daniel et al. 2007b
	Altered lamellar morphology of lumbar discs	Gruber et al. 2008
	Altered cartilage/bone ratio during bone fracture healing	Taylor et al. 2009
	Reduced notch3 target gene expression	Meng et al. 2009
	Increased age-related dilated cardiomyopathy and age-related mortality	Swinnen et al. 2009
	Inhibition of adipogenesis	Shitaye et al. 2010

Continued

Table 6. *Continued*

TSP	Mouse gene knockout phenotype	Reference
TSP-3	Transient alteration of biomechanical properties of bone (PN weeks 9–15) Accelerated ossification of the head of the femoral bone	Hankenson et al. 2005b
TSP-4	No overt phenotype. *Thbs4*$^{-/-}$; *ApoE*$^{-/-}$ mice have reduced development of atherosclerotic lesions and reduced vascular inflammation in lesions	Frolova et al. 2010
TSP-5/COMP	No detectable skeletal phenotype in unchallenged mice Altered growth plate organization	Svensson et al. 2002 Posey et al. 2008

See text for discussion of additional phenotypes in mice that lack multiple TSP family members.

myocardial infarction, renal fibrosis, diabetes, experimental autoimmune uveoretinitis, and tumor progression (Table 6). Inflammatory cell recruitment and fibrosis are decreased during these processes in *Thbs1*$^{-/-}$ mice. The immune privilege of retinal pigment epithelial cells is maintained by TSP-1-mediated activation of TGFβ (Zamiri et al. 2005). Some disease phenotypes in *Thbs2*$^{-/-}$ mice are related to aging and indeed *Thbs2*$^{-/-}$ mice display increased age-related mortality (Table 6). Collectively, these results highlight the complexity of interpreting the diverse phenotypes of TSP-deficient mice: in the absence of TSP-1 or TSP-2 an extensive network of interacting proteins are disrupted with multiple tissue-specific phenotypic consequences.

Roles in Mouse Cancer Models

In general, tumor cells down-regulate TSP-1 expression to promote angiogenesis (Ren et al. 2006). This endpoint is achieved by multiple mechanisms, including: (1) secretion of soluble factors that down-regulate TSP-1 in surrounding fibroblasts (Kalas et al. 2005), (2) loss of TSP-1-dependent inhibition of tumor growth or endothelial cell migration by TGFβ activation (Miao et al. 2001, Motegi et al. 2008), (3) inhibition of VEGF mobilization from the extracellular matrix by MMP-9 (Rodriguez-Manzaneque et al. 2001), (4) down-regulation of circulating endothelial cell progenitors (Shaked at al. 2005), (5) induction of endothelial cell apoptosis (Jimenez et al. 2000), and (6) suppression of melanoma growth by recruitment of M1 macrophages and innate antitumor immunity (Martin-Manso et al. 2008). Megakaryocytes and platelets represent key sources of TSP-1 that regulate bone marrow and tumor angiogenesis (Kopp et al. 2006; Zaslavsky et al. 2010). The tumor-suppressive role of TSP-1 is supported by findings that, in the absence of TSP-1, tumors progress more rapidly in *neu/erbB2*, *APC*$^{Min/+}$, and p53-deficient mouse models (Lawler et al. 2001; Rodriguez-Manzaneque et al. 2001; Gutierrez et al. 2003).

TSP-1 is reported to both inhibit and stimulate metastasis. Inhibitory effects in some cancer models are probably secondary to inhibition of angiogenesis (Weinstat-Saslow et al. 1994; Hawighorst et al. 2001). Because lymphatic vessels have little or no CD36 in vivo, TSP-1 does not inhibit tumor-associated lymphangiogenesis or tumor cell spread to regional lymph nodes (Hawighorst et al. 2001). TSP-1 also reportedly mediates the antimetastatic effect of prosaposin, the precursor form of the lipid hydroxylase activators saposin A-D (Kang et al. 2009). Contrary to these results, TSP-1 promotes metastasis in a transgenic model of breast cancer, likely because of promotion of cell migration (Yee et al. 2009). Migration of invasive breast, melanoma, or thyroid cancer cell lines is also promoted by TSP-1 in vitro (Wang et al. 1996; Albo et al. 1998; Nucera et al. 2010). In several cases, increased migration correlates with elevated activity of extracellular proteases (Albo et al. 1998; Liu et al. 2009). These data further underscore the pleiotropic activities of TSP-1 in cell–ECM

interactions and the differential responses of various cell types, resulting in multifaceted effects on tumor progression.

Data from Humans

TSP-5/COMP and PSACH

COMP/THBS5 is the causal gene for PSACH ([Online Mendelian Inheritance in Man—OMIM177170] Newton et al. 1994; Briggs et al. 1995; Hecht et al. 1995). Individuals heterozygous for a mutant allele have shortened stature, joint laxity, joint erosion and pain, and early onset osteoarthritis. Subsequent studies have shown that: (1) PSACH mutations occur in multiple locations of the coding sequence of *COMP/THBS5*, and (2) mutations can also lead to EDM1 (OMIM132400) (Posey and Hecht 2008). Because mutations of collagen IX or matrilin-3 also lead to forms of EDM, the three proteins might work in concert during cartilage ECM assembly.

In PSACH patients and, to a lesser extent, EDM patients, the ER of chondrocytes is dilated with alternating electron-dense and electron-lucent layers that contain collagen II, TSP-5/COMP, collagen IX, matrilin-3, aggrecan, and other ECM proteins (Briggs and Chapman 2002; Merritt et al. 2007). Pulse-chase experiments indicate that many TSP-5/COMP mutants are secreted less rapidly than wild type (Chen et al. 2008). The increased transit time in the ER results in increased interactions between the ECM proteins leading to formation of inclusions and ER stress, chondrocyte death, and premature slowing of bone growth. Some TSP-5/COMP mutations have less severe effects on protein secretion and these may affect extracellular functions of TSP-5/COMP.

Because gene deletion of *Thbs5/COMP* has minor phenotypic consequences in mice (Table 6), the concept of silencing TSP-5/COMP expression, to reduce the burden of mutant TSP-5/COMP in chondrocytes, is gaining interest as a possible therapeutic strategy. A hammerhead ribozyme against the common D569del mutation significantly reduces mutant TSP-5/COMP mRNA levels in chondrocytes (Alcorn et al. 2009). Reduced TSP-5/COMP levels, ER stress, and intracellular retention of other ECM proteins have been achieved with short hairpin RNA against TSP-5/COMP in cultured cells (Posey et al. 2010).

TSP Single Nucleotide Polymorphisms and Disease

Single nucleotide polymorphisms (SNPs) in TSP-1, -2, and -4 correlate with increased risk of premature myocardial infarction (Topol et al. 2001). However, a recent meta-analysis failed to detect significant correlations (Koch et al. 2008). Nevertheless, biochemical and cellular analyses of the N700S and A387P SNPs of TSP-1 and TSP-4, respectively, have identified effects on calcium binding, protein conformation, and interactions with cells and ECM components of the vessel wall and platelet clot (Stenina et al. 2003, 2005; Narizhneva et al. 2004; Carlson et al. 2008b). It is possible that subtle differences in the patient populations may account for the discrepancies in clinical correlations. For example, the TSP-2 SNP correlates with cases that involved plaque erosion (Burke et al. 2010). Another TSP-2 SNP affects skipping of exon 11 and correlates with lumbar-disc herniation in the Japanese population. Exon 11 encodes the third TSR of TSP-2 and without this TSR, TSP-2 has reduced binding to MMPs (Hirose et al. 2008).

TRANSLATIONAL APPLICATIONS

TSR Domains, Angiogenesis, and Cancer

Therapeutic strategies to exploit the antiangiogenic activity of TSP-1 and -2 have become of great interest (reviewed by Zhang and Lawler 2007). Approaches demonstrating significant efficacy in mouse preclinical models include the delivery of synthetic peptides or recombinant proteins through direct injection, adeno-associated viruses, or cells. A peptide mimetic, ABT-510, based on the second strand of the second TSR, was taken to phase II clinical trials by Abbott Laboratories; however, as a single agent, ABT-510 did not have significant clinical efficacy against metastatic melanoma and renal cell carcinoma (Westphal 2004; Ebbinghaus

et al. 2007; Markovic et al. 2007). Recombinant proteins that also include the RFK sequence that activates TGFβ1 (see section Major Binding Partners), have increased antitumor activity in a mouse model (Miao et al. 2001; Yee et al. 2009). Cell-based strategies to deliver intact TSP-1, TSP-2, or the TSRs have been developed and have provided effective inhibition of several experimental cancers (Streit et al. 2002; van Eekelen et al. 2010). ABT-510 improved the uptake and efficacy of cisplatin and paclitaxel in a mouse ovarian cancer model (Campbell et al. 2010), and its activity was increased in combination with troglitazone, which up-regulates CD36 expression on endothelial cells (Huang et al. 2004). The TSRs of TSP-1 also increase the antiangiogenic activity of TRAIL to inhibit colon cancer in a mouse subcutaneous model (Ren et al. 2009). Thus, the TSRs may have important applications in combination cancer therapy. A small molecule mimetic of the FGF-2 binding site of TSP-1 is also in development as a potential inhibitor of angiogenesis (Margosio et al. 2008; Colombo et al. 2010).

TSP-5/COMP Oligomerization Domain

The TSP-5/COMP coiled-coil domain has been used to create engineered pentameric chimeras of bioactive molecules with enhanced stability and improved properties to activate or inhibit specific signaling pathways (Holler et al. 2000; Cho et al. 2004; Wang et al. 2008). Activation of nonphysiological receptors, such as Tie2 receptor by an engineered angiopoietin-2 pentamer, has also been achieved (Kim et al. 2009). The TSP-5/COMP pentamerizing coiled-coil has wide potential for development of high-affinity or stable ligands for clinical or bioengineering applications.

FUTURE DEVELOPMENTS

There are many areas of TSP biology that remain to be explored and translational areas that are expanding. Key questions and developing areas include:

- The relationship of structure to function in the TSP carboxy-terminal region, with benefit of the universe of TSP sequences from invertebrates.
- The mechanisms and roles of TSPs in collagen fibril organization.
- The roles of TSPs in calcium homeostasis.
- The cell biology of pentameric TSPs.
- The functions of TSPs in invertebrates, especially within the ECMs of Cnidaria and sponges.
- The conserved roles of TSPs in the ovary and their relevance to fertilization mechanisms.
- The roles of TSPs in excitatory synaptogenesis, and their relevance to learning, memory, and pain perception throughout life.
- The mechanisms and potential biological significance of bacterial adhesion to TSPs.
- The feasibility and practicality of TSPs/TSP interactions as therapeutic targets, especially in cancer development and metastasis, cardiovascular disease, fibrosis, and ischemia.
- The application of engineered TSP moieties in designed molecules or synthetic cellular environments.

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