The face of TSR revealed: an extracellular signaling domain is exposed

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In this issue, Tan et al. (2002) report the first high resolution (1.9 Å) structural data for thrombospondin (TSP)-1, a large multifunctional protein that regulates cell adhesion, angiogenesis, cell proliferation and survival, TGFβ activation, and protease function (for review see Chen et al., 2000). Because TSP-1 has multiple binding partners and many functions, precise structural information is crucial to understanding its biology. The structure now reported, derived from crystals of the second and third type I repeats of TSP-1 is of particular interest because of the specific functions attributed to these repeats and because domains homologous to the repeats appear in many other proteins in nature. The novel layered fold motif described brings great insight into how the complicated functions of TSP-1 and related molecules are affected.

TSP-1 is the prototypical member of a group of secreted, extracellular matrix (ECM)* proteins referred to as matricellular proteins (Bornstein, 1995). This name signifies their major role as matrix-based cellular signaling molecules rather than as determinants of matrix structure. These proteins are deposited in the matrix in a highly regulated manner, e.g., during embryonic development or postnatal remodeling events such as wound healing, and function via specific interactions with growth factors, proteases, and receptors on migrating cells. TSP-1, a 450-Kd homotrimer was first identified as a protein secreted in large amounts from the α-granules of activated platelets. Platelet TSP is easily purified and has been very well studied. In vitro experiments have identified numerous binding partners, including fibrinogen, fibronectin, plasminogen, thrombin, elastase, matrix metalloproteases, heparin, sulfated glycolipids, and cellular surfaces (Chen et al., 2000). Despite seemingly critical homeostatic roles, the phenotype of the murine TSP-1 knock-out strain is subtle (Lawler et al., 1998), perhaps the result of redundancies among the matricellular proteins as a group.

The myriad effects of TSP-1 on cellular functions may seem confusing and inconsistent. For example, TSP-1 has been reported in some studies to promote cell adhesion, cell proliferation, angiogenesis, and tumor progression, whereas other equally compelling studies show disruption of cell adhesion, induction of apoptosis, inhibition of angiogenesis, and inhibition of tumor growth. These inconsistencies, however, are readily explainable by its ability to interact specifically with a range of cellular receptors expressed differentially by different cell types. These include at least three integrins (α3β1, αvβ3, and αIIbβ3), CD47 (integrin-associated protein), CD36, LDL receptor–related protein, and cellular glycosaminoglycans (GAGs). Also, TSP-1 has been shown to regulate protease and growth factor functions, adding a further level of complexity to its effects on cells.

Understanding of the complex biological functions of TSP-1 followed to a large extent from analysis of its structure. Initial studies using limited proteolysis and SDS-PAGE suggested a divalent cation-dependent modular structure, similar to many other proteins of the ECM. This was confirmed with the generation and mapping of a series of monoclonal antibodies. Rotary shadowing electron microscopy revealed that the monomers were tethered at large amounts from the α-granules of activated platelets. Platelet TSP is easily purified and has been very well studied. In vitro experiments have identified numerous binding partners, including fibrinogen, fibronectin, plasminogen, thrombin, elastase, matrix metalloproteases, heparin, sulfated glycolipids, and cellular surfaces (Chen et al., 2000). Despite seemingly critical homeostatic roles, the phenotype of the murine TSP-1 knock-out strain is subtle (Lawler et al., 1998), perhaps the result of redundancies among the matricellular proteins as a group.

*Abbreviations used in this paper: ECM, extracellular matrix; GAG, glycosaminoglycans; HB-GAM, heparin binding growth-associated molecule.

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components of the complement system and in the circum-
sporozoite protein encoded by the genome of the unicellular
parasite falciparum malaria. Comparative genomics now re-
veals that the primordial exon encoding the type I repeat is
ancient and has been extensively duplicated and shuffled
during evolution; >40 human genes contain one or more
copies of the repeat, along with >10 in the fly, 20 in the
worm, and 2 in falciparum malaria. Among these are the
ADAM-TS family of metalloproteinases, F- and M-spon-
dins, semaphorins, Unc5, heparin binding growth-associ-
mated molecule (HB-GAM), and brain angiogenesis inhibitor
(BAI)-1. Of those whose functions have been probed, most
seem to be involved in cell matrix interactions and in the
control of migration, axonal guidance, and/or matrix re-
modeling during development (Adams and Tucker, 2000).
It is the type I repeat in many of these proteins that seems to
be a critical mediator of cell–matrix interactions.

The type I/properdin repeat has now been renamed TSR
(thrombospondin structural homology repeat). It is ~60
amino acids in length, ≥12 of which are highly conserved,
including 2–3 tryptophans, 5–6 cysteines, and 2 arginines.
Solution spectroscopic studies of a TSR from distant TSP
“cousins” HB-GAM and midkine were reported in 2000
(Kilpelainen et al., 2000) and suggested an elongated struc-
ture with a large surface area. Tan et al. have now success-
fully crystallized the second and third TSR from TSP-1 and
solved the structure at high resolution. This work sheds con-
siderable light on the often confusing and paradoxical biol-
y of TSR-1.

Their studies show that TSR is a highly structured do-
main organized as a long right-handed spiraling ribbon con-
taining three antiparallel strands (two are β sheets and the
third is a less structured “rippled” strand). The most novel
aspect of the structure is that the three antiparallel strands
are held together by a series of interlocking stacks of amino
acid side chains made up of six alternating layers of trypt-
ophans (W layer) and arginines (R layer) sandwiched be-
tween cystine disulfides on the top and bottom. This pattern
of stacked planar cationic guanidium groups and aromatic
groups is stabilized by multiple cation-π interactions and
forms a continuously positive charged face containing a
groove-like structure that could easily be imagined forming a
recognition site for ligands. Several testable hypotheses thus
emerge from this work. The authors speculate that an an-
ionic disaccharide unit from a right-handed spiraling hepar-
in molecule could “fit” well into the cationic groove formed
in the 20-Å distance between the first W layer and the third
R layer. It is tempting to speculate that the degree of positive
charge and shape of the groove within the charged face of
the domain along with the number of sequential repeats may
regulate the specificity and affinity for GAGs. ADAM-TS4
(aggrecanase) and F-spondin have additional arginines in the
C strand of some of their TSRs and thus a more positive
charged face than TSP-1. F-spondin and Sema5 have a
larger number (seven) of sequential TSRs. Whether these
features relate to the ability of ADAM-TSY to bind and spe-
cifically cleave aggrecan or of F-spondin to guide neurite
outgrowth on the developing floor plate, remains to be seen.

Another role for the TSR in TSP-1 and -2 is as a ligand
for the cell surface receptor CD36 (Asch et al., 1987). This
interaction, although initially controversial, is of consid-
erable interest in that it has now been shown convincingly to
mediate the antiangiogenic, proapoptotic effects of TSP on
microvascular endothelial cells (Dawson et al., 1997). Sev-
eral other TSR proteins are also antiangiogenic, although it
is not yet known whether all operate via CD36, nor are the
structural factors known that determine whether a TSR pro-
tein binds CD36. Tan et al. have divided the TSR proteins
into two large groups based on putative structural differ-
ences, including the position of the first cystine layer. Al-
though data are not available for all, the known antiangi-
genic members are all in one of the groups. Studies using
synthetic peptides have suggested that the conserved CS-
VTCG sequence in TSRs is the likely binding site for CD36
(Asch et al., 1992); CSVVTCG peptides inhibit angiogenesis
and block TSP-1 binding to CD36-expressing cells. More
difficult to explain are studies showing that peptides con-
taining sequences flanking, but not including, CSVVTCG
are also antiangiogenic (Tolksma et al., 1993). In fact a
D-isoleucine enantiomer of the highly conserved GVITRIR
sequence is nearly as potent as TSP-1 itself and is being de-
veloped as an antiangiogenesis-based anticancer drug. The
crystal structure of TSR reveals that the cationic GVITRIR
sequence makes up part of the positively charged face of the
domain, whereas the CSVTCG sequence is not readily avail-
able with its two cysteines involved in separate disulfide
bonds. The hypothesis that the cationic face of the TSR
serves as a CD36 ligand is consistent with work from our
laboratory and others showing that the binding site on
CD36 for TSP is between amino acids 90 and 110 (Pearce
et al., 1995) and contains a negatively charged sequence that
is conserved in murine CD36. Furthermore, this sequence is
conserved in CD36 homologous domains found in other
TSP binding proteins, including histidine-rich glycoprotein,
LIMP2, and HIV gp120 (Crombie and Silverstein, 1998).
Cocrystallization of the TSR with its binding domain from
CD36 or CD36 homologues is the next challenge and may
allow us to establish parameters to predict which TSR pro-
teins are likely to be antiangiogenic.

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