Meet the Tenascins: Multifunctional and Mysterious*

Henry C. Hsia and Jean E. Schwarzbauer‡
From the Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

The tenascins are a highly conserved family of large oligomeric glycoproteins found in the extracellular matrix (ECM) of vertebrate organisms. Two decades ago, the molecule now known as tenascin-C was among the first proteins shown to have an adhesion modulatory role antagonizing cell attachment to fibronectin (1). Cells that normally display a stationary phenotype on the fibronectin-containing matrix by spreading out and forming cortical actin stress fibers (Fig. 1A) will show morphological changes when tenascin-C is included in the matrix by extending membrane protrusions and exhibiting decreased stress fiber formation (Fig. 1B), characteristics more typical of a migratory phenotype (2). The presence of ECM proteins such as tenascins during developmental and pathological states is now well recognized if still not well understood. Although each type has a distinctive expression pattern, the tenascins share the characteristic of having tightly regulated expression during development and throughout an organism’s life. Tenascins also share the characteristic of modulating cell-matrix interactions and mediating a state of matrix attachment that promotes motility while also influencing other cell functions. As such, this protein family has important functions not only during development but also during pathological states in the adult such as tissue injury and tumorigenesis where remodeling processes are prominent. Reviews in recent years have covered pertinent aspects of tenasin biology including domain structure, modulation of cell functions, in vivo null deletion phenotypes, and contributions to human pathology (3–10). This minireview will provide a concise summary of information from these reviews, which the reader is encouraged to refer to for greater detail, and will also discuss a few of the more recent developments in the field.

About the Family

Nomenclature and Structure—Tenascins are considered unique to vertebrates. Although Drosophila proteins such as ten-m/Odz were once thought to be possible orthologues due to shared motifs, the identification of more closely related vertebrate equivalents has left the tenascin family without any known invertebrate orthologue (11, 12). There are currently four tenasin paralogues that have been identified in mammals, each designated with a letter derived, for the most part, from earlier eponyms: C, R, X, and W. The primary structures of tenasin family proteins have common motifs all ordered in the same consecutive sequence (Fig. 2); amino-terminal heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III domain repeats, and a carboxyl-terminal fibronogen-like globular domain. The heptad repeats lie in a highly conserved amino-terminal oligomerization region allowing individual subunits to assemble, usually into trimers. In some tenascins, additional cysteine residues allow the assembly of two trimers into a hexamer. Each protein member is associated with typical variations among different species in the number and nature of EGF-like and fibronectin type III repeats. Isoform variants produced through alternative splicing within the fibronectin type III repeats have been described across the family; however to date, only tenascin-C has shown splice variants being expressed in significant numbers and diversity (5, 7).

Tenasin-C—Assembly into hexamers is a classic feature of tenasin-C. Glycosylation differences can cause the sizes of individual subunits to be quite variable, but most are generally around 200 kDa, although some human tenasin-C monomers have been estimated to be over 300 kDa (5, 9). Mammalian protein subunits typically have 14.5 EGF-like repeats with 8 fibronectin type III repeats that are shared by all tenasin-C isoforms. Alternative splicing of an additional 9 distinctive repeats that can be independently included or excluded in a combinatorial manner allows tenasin-C to show the greatest number and diversity in isoforms with as many as 27 different mRNA variants having been identified in the developing mouse brain (13). Although much data exist demonstrating that splice variants are differentially expressed during tissue morphogenesis and tumorigenesis under the influence of growth factors and cytokines such as transforming growth factor-β and basic fibroblast growth factor, the determining mechanisms underlying tenasin-C alternative splicing are still not known (9). As the first tenasin to be identified, tenasin-C remains the best studied member of the family, accounting for most reports examining the component domains shared by tenasin family proteins. These domains and their functions, however, also remain poorly understood. Although it is believed that the different regions of tenasin-C have distinct actions and functions, it is also probable that the overall effects of tenasin-C on cells and their interactions with the ECM require the concerted action of multiple domains (14). A multiplicity of binding sites have been identified for integrin cell surface receptors, proteoglycans, and cell adhesion molecules of the immunoglobulin family, as well as annexin II receptor proteins and ECM components such as heparin, fibronectin, and collagen. Almost all known binding sites lie in the fibronectin type III repeats or the fibrinogen globule, but evidence has been reported suggesting that the EGF-like repeats act as low affinity ligands for EGF receptors (15). As a consequence, cell interactions with tenasin-C are quite complex, and several signaling mechanisms have been identified, including recent observations that tenasin-C blocks focal adhesion kinase- and Rho-mediated signaling pathways activated by fibronectin (2, 16, 17) as well as stimulating Wnt and other growth-promoting pathways (18). For a detailed discussion of these mechanisms, please refer to Gertrude Orend’s recent review (4).

In the developing embryo, tenasin-C is expressed during neural, skeletal, and vascular morphogenesis. It then virtually disappears in the adult organism with continued basal expression detectable only in tendon-associated tissues; however,

* This minireview will be reprinted in the 2005 Minireview Compendium, which will be available in January, 2006.
‡ To whom correspondence should be addressed. Tel.: 609-258-2893; Fax: 609-258-1035; E-mail: jschwartzbauer@molbio.princeton.edu.
1 The abbreviations used are: ECM, extracellular matrix; EGF, epidermal growth factor.
sharp up-regulation in expression occurs in tissues undergoing remodeling processes seen during wound repair and neovascularization or in pathological states such as inflammation or tumorigenesis. Given this expression pattern and that its structure has been well conserved among vertebrates and appears to be capable of numerous interactions and potential functions, reports during the 1990s demonstrating that mice with complete tenascin-C gene deletions are viable and appear to develop normally were unexpected (19, 20). Later reports have since noted more subtle abnormalities, and the issue of tenascin knock-out phenotypes will be discussed further below.

Tenascin-R—Having subunits 160–180 kDa in size that can form oligomers of 2 or 3 polypeptide chains, tenascin-R shares a high degree of structural homology with tenascin-C. Its expression seems limited exclusively to the central nervous system, although one report of expression in a cell line originating from the peripheral nervous system has been published (21). Subunits typically have 4.5 EGF-like repeats and 8–9 fibronectin type III repeats. Alternative splicing at the sixth fibronectin type III repeat produces two isoforms that, even when compared with other tenascins, are very highly conserved across different species (3, 22). Although the 160-kDa isoform tends to form dimers and the larger 180-kDa isoform trimers, the functional significance of the two isoforms is not understood (3). Tenascin-R expression has some degree of overlap with tenascin-C expression in the developing nervous system, but unlike tenascin-C, the onset of tenascin-R expression occurs at later time points (23). In vitro studies have demonstrated that the protein influences neural pattern formation through adhesive and anti-adhesive effects on cell-matrix interactions (3); however, like their tenascin-C counterparts, tenascin-R knock-out mice are viable and fertile and only recently has it been demonstrated that they also show behavioral differences (24–26).

Tenascin-X—Over 400 kDa in size, tenascin-X is the largest known member of the family and widely expressed during development. Adult expression, however, is mostly limited to musculoskeletal, cardiac, and dermis tissue. Individual subunits have 18.5 EGF-like repeats and 29 or more fibronectin type III repeats depending on the species (27–29). Although tenascin-X appears capable of forming trimers, it differs from other family members in that it lacks the amino-terminal cysteine residues involved in hexamer formation. Although alternative splicing of the fibronectin type III repeats has not been described for tenascin-X in humans, splice variants have been reported for the mouse homologue (28, 30, 31). A close relative sharing similar expression patterns, but smaller in size (170–220 kDa subunits) with significantly fewer EGF-like repeats, was originally identified in chickens as a new tenascin parologue called tenascin-Y (32). It contains among the fibronectin type III repeats a region rich in amino acid triplets of serine and proline, an unusual domain that has been found in some variants of tenascin-X (5). More recently, although the two proteins differ greatly in their respective numbers of EGF-like repeats, their many other similarities have led to the suggestion that tenascin-Y is an avian orthologue of tenascin-X (6, 7).

Tenascin-X is the first tenascin whose deficiency has been clearly associated with a pathological disorder in humans, a variant of a heritable connective tissue disorder known as Ehler-Danlos Syndrome, which is associated with fibrillar collagen defects. The tenascin-X gene overlaps on human chromosome 6 with the gene coding for the steroid 21-hydroxylase whose deficiency results in congenital adrenal hyperplasia. The first instances of human tenascin-X deficiency were found in patients exhibiting clinical signs of both congenital adrenal hyperplasia and Ehler-Danlos Syndrome who were found to have a contiguous deletion encompassing both genes (33, 34).

Tenascin-W—First identified in zebrafish (35), tenascin-W remains the least well characterized member of the tenascin family. Trimers of 130-kDa subunits have been isolated from zebrafish tissues with each subunit containing 3.5 EGF-like repeats and 5 fibronectin type III repeats. Isoforms from alternative splicing have not been reported for zebrafish tenascin-W. Expression has been found in developing skeletal tissue and neural crest cells with a pattern that partially overlaps tenascin-C expression patterns. Although descriptions of tenascin-W expression have been almost exclusively limited to zebrafish, recent reports have identified possible mammalian orthologues (see below and Fig. 2).
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Tenascin biology seems to be embedded in a web of complexity that mirrors human biology as a whole. The multifunctional nature of tenascins seems obvious from the shared similarities of their repeating domains with proteins such as EGF, fibronectin, and fibrinogen. Their highly conserved structures and tightly regulated expression patterns only reinforce the perception that tenascins must have some critical purpose in vertebrate biology. The inability to identify any clear and specific role, however, has led to tenascins being labeled over a decade ago as “talented proteins in search of functions” (12). Although a great deal of knowledge has been gained about these proteins in the interim including an association with a specific human disease, they remain frustratingly mysterious entities whose specific functions are still not well understood. Nonetheless, it is evident that tenascins represent an important class of extracellular proteins that have a clear ability to regulate cell behavior and therefore carry significant ramifications in our understanding and treatment of a broad and diverse range of human disease.

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