Transcriptional regulation of tenasin genes

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Abbreviations: AKT, v-akt murine thymoma viral oncogene homolog; ALK, anaplastic lymphoma kinase; ATF, activating transcription factor; AP-1, activator protein-1; BMP, bone morphogenetic protein; CBP, CREB binding protein; ChIP, chromatin immunoprecipitation; CREB, cAMP response element-binding protein; CREB-RP, CREB-related protein; CYP21A2, cytochrome P450 family 21 subfamily A polypeptide 2; EBS, Ets binding site; ECM, extracellular matrix; EGF, epidermal growth factor; ERK1/2, extracellular signal-regulated kinase 1/2; ETS, E26 transformation-specific; Evx1, even skipped homeobox 1; FGF, fibroblast growth factor; HBS, homeobox domain binding sequence; IL, interleukin; ILK, integrin-linked kinase; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MHCIII, major histocompatibility complex class III; miR, micro RNA; MKL1, megakaryoblastic leukemia-1; NGF, nerve growth factor; NFAT, nuclear factor of activated T-cells; NFκB, nuclear factor kappa B; OTX2, orthodenticle homolog 2; p38 MAPK, p38 mitogen activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; POU3F2, POU domain class 3 transcription factor 2; PRRX1, paired-related homeobox 1; RBPjk, recombining binding protein suppressor of hairy; RhoA, ras homolog gene family member A; ROCK, Rho-associated, coiled-coil-containing protein kinase; SAP, SAF-A/B, Acinus, and PIAS; SCX, scleraxis; SEAP, secreted alkaline phosphatase; SMAD, small body size - mothers against decapentaplegic; SOX4, sex determining region Y-box 4; SRE, serum response element; SRF, serum response factor; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-β; TNC, tenascin-C; TNF-α, tumor necrosis factor-α; TNR, tenasin-R; TNW, tenasin-W; TXN, tenasin-X; TSS, transcription start site; UTR, untranslated region; WNT, wingless-related integration site.

Extracellular matrix proteins of the tenasin family resemble each other in their domain structure, and also share functions in modulating cell adhesion and cellular responses to growth factors. Despite these common features, the 4 vertebrate tenascins exhibit vastly different expression patterns. Tenasin-R is specific to the central nervous system. Tenasin-C is an “oncofetal” protein controlled by many stimuli (growth factors, cytokines, mechanical stress), but with restricted occurrence in space and time. In contrast, tenasin-X is a constitutive component of connective tissues, and its level is barely affected by external factors. Finally, the expression of tenasin-W is similar to that of tenasin-C but even more limited. In accordance with their highly regulated expression, the promoters of the tenasin-C and -W genes contain TATA boxes, whereas those of the other 2 tenascins do not. This article summarizes what is currently known about the complex transcriptional regulation of the 4 tenasin genes in development and disease.

Introduction: The Tenasin Gene Family

Tenascins are a family of large, oligomeric, multi-domain extracellular matrix (ECM) proteins.1 Four genes encoding tenasin-C (TNC), tenasin-R (TNR), tenasin-X (TXN), and tenasin-W (TNW) proteins exist in higher vertebrates, and a single tenasin gene is found in cephalochordates whereas similar genes and proteins do not seem to exist in other animal phyla.2,4 Tenascins are characterized by their unique domain structure. Each monomeric unit comprises an N-terminus with heptad repeats flanked by cysteine residues. This N-terminal oligomerization region is followed by EGF-like repeats, and a variable number of fibronectin type III repeats as a result of alternative mRNA splicing. At the C-terminus, each subunit ends with a large C-terminal fibrinogen related domain.1 Via their N-termini, tenasin subunits form disulfide-linked homo-trimers (TNR and TNX) or -hexamers (“hexabrachions;” TNC and TNW). Rather than representing bona fide structural components of the extracellular matrix, tenascins are “matricellular” proteins involved in modifying the interaction of cells with extracellular matrix and growth factors, and hence regulating cell adhesion, migration, growth and differentiation in a context-dependent manner (see other articles in this issue).

A number of earlier reviews have summarized the discovery,6–9 protein structure,1,8 splice variants,10,11 binding partners and cellular receptors,12 expression patterns13 and functions in vitro and in vivo9,14–17 of the 4 tenascins, and more information on these topics is to be found in other contributions to this special issue. The present article has a different and narrow focus, namely to summarize what is currently known about the regulation of expression of tenascins. We briefly review the expression patterns...
of the 4 tenascins in development, regeneration and disease, and focus on the transcriptional regulation of their respective genes by growth factors and mechanical stimuli. Except for TNX, which has a widespread distribution like many ECM proteins, the other 3 tenascins show a very restricted occurrence during embryogenesis, tissue remodeling and tumor formation.\textsuperscript{5,9,18,19} Their patterns of localization, which are specific for each of the 4 family members, point to tightly controlled spatial and temporal expression, and are likely to reflect complex gene regulation. To date, the promoter of the \textit{TNC} gene has been studied most extensively in various species, whereas information on the gene promoters of the other 3 family members is still comparatively sparse. Thus, the apparent imbalance between chapters in this article reflects the current status of the literature.

**Tenascin-C: Expression in Organogenesis, Inflammation, Tissue Repair and Cancer**

**Structure of the tenascin-C (TNC) gene**

Tenascin-C (gene name \textit{TNC}) is the founding member of the respective family of ECM proteins.\textsuperscript{5} The human \textit{TNC} gene (gene ID: 3371) is on chromosome 9q33; it contains 29 exons of which 9 (each coding for a fibronectin type III domain) can be alternatively spliced.\textsuperscript{20-22} The transcript starts with a non-coding exon, separated by an intron >20 kb long, and followed by exon 2, which contains the ATG start codon for translation initiation. \textit{TNC} mRNA from human fibroblasts and human melanoma cells analyzed by primer extension and S1 nuclease showed a single transcription start site (TSS) localized to the first exon (Fig. 1). Sequencing of approximately 2300 bp of the \textit{TNC} gene 5'-flanking region has revealed several potential binding sites for transcription factors (see below).\textsuperscript{20} The sequence of 220 bp upstream to the TSS was identified as region with high promoter activity; it contains a classical TATA box at $-20$ to $-26$ bp. A putative silencer sequence was localized to the fragment between $-220$ and $-2300$ bp.\textsuperscript{20} Similarly, primer extension analysis of mRNA isolated from brain tissue of mouse embryos showed a single TSS that lays 27 bp downstream of the TATA box.\textsuperscript{23} Moreover, the 230 bp proximal promoter sequence, which is conserved between species, was found to be highly active in driving reporter gene expression when transfected into both mouse and human fibroblasts.\textsuperscript{23} The chicken \textit{TNC} (cytotactin) gene features a TATA box at a similar position as the mammalian counterparts.\textsuperscript{24} A comparison between the human, mouse and chicken \textit{TNC} promoters has been presented by Jones and Jones (2000).\textsuperscript{25}

**Figure 1.** Schematic representation of all tenascin genes. Gene models of \textit{TNC}, tenascin-C; \textit{TNW}, tenascin-W; \textit{TNR}, tenascin-R; \textit{TNXB}, tenascin-X were captured from the NCBI database (http://www.ncbi.nlm.nih.gov/\textit{gene/}). \textit{TNC}, \textit{TNW} and \textit{TNR} have a single transcription start site (TSS1) whereas the \textit{TNXB} gene has 4 closely clustered TSSs(TSS1–4) in its promoter shown here. Non-coding exons up to the first coding exons (indicated by the translation start codon ATG) as well as the last exons are numbered with e1, 2, . . . below the models. Note that the \textit{TNC} and \textit{TNN} genes possess TATA boxes (red triangles) whereas the \textit{TNR} and the \textit{TNXB} genes do not.

**Tenascin-C gene regulation by patterning genes during development**

\textit{TNC} received much attention after its discovery because of its highly specific and restricted expression patterns during vertebrate embryogenesis.\textsuperscript{26,27} In contrast to many other ECM proteins, \textit{TNC} often appears in an all-or-none fashion both in space and time. Specifically, the protein is an early marker of tendon, ligament and bone formation.\textsuperscript{26} Other prominent sources are neural crest cells in early embryos,\textsuperscript{28} Schwann cell precursors in developing peripheral nerves,\textsuperscript{29,30} and vascular smooth muscle cells around arteries.\textsuperscript{31} In addition, \textit{TNC} expression is often associated with specific morphogenetic events during organogenesis, e.g. with the formation of somites, segmental nerves,\textsuperscript{30} mammary glands,\textsuperscript{27,32} teeth,\textsuperscript{33} kidneys\textsuperscript{34} and lungs.\textsuperscript{35} It was therefore an obvious possibility that the \textit{TNC} gene could be controlled by segmentation and patterning genes. Indeed, some of the early publications on \textit{TNC} promoters from different species investigated their regulation by homeobox transcription factors (for detailed information about the location and sequence of cis-acting elements in the \textit{TNC} promoter, see Table 1 and Fig. 2). For example, the chicken promoter was found to be strongly activated by co-transfection of fibroblasts with even-skipped homeobox 1 (EVX1), and by mutational analysis, an AP1 element was identified that was essential for this response.\textsuperscript{36} The same AP1 site was found to mediate activation of the promoter by serum growth factors, and EVX1 overexpression potentiated the effect of serum. Thus, EVX1 appears to activate the \textit{TNC} gene indirectly by synergizing with JUN/FOS transcription factors, which target the AP1 site.

On the other hand, a homeobox transcription factor involved in anterior head formation, orthodenticle homolog 2 (OTX2), was shown to bind directly and with high affinity to the human \textit{TNC} promoter and to suppress its transcriptional activity;\textsuperscript{37,38} the OTX2 target sequence is conserved in the mouse (but not chicken) gene. Similarly, the POU-homeodomain transcription factor POU3F2 (also called BRN2 or Oct-7) was demonstrated to interact directly with a reverse octamer sequence in the mouse.
Table 1. Summary of transcriptional regulation of the tenascin gene promoters. For each publication cited, the species of the promoter analyzed, the transcription factor(s) over-expressed or growth factors added, the response elements and their sequences and relative position to the TSS are listed together with a short description of the main experimental evidence provided.

| Gene | Promoter species | Transcription factor overexpressed or factors added | Response element | Sequence | Position | Functional assays |
|------|------------------|----------------------------------------------------|-----------------|----------|----------|-------------------|
| TNC  | Chicken          | 24                                                  | TRE/AP1         | CTGAGTCAT| −3986 to +121 | 3986bp more active than 721bp promoter in chicken embryo fibroblasts. 3986bp promoter active in human U251MG cells, but not in HT1080 cells. |
| Chicken | EVX1 (mouse)     | ETS1                                                | CTGAGTCAT       | −281 to −273 | Reporter activation in NIH3T3 cells and inactivation by mutation of response elements. |
| Chicken | HBS 1            | ETS1                                                | CTGAGTCAT       | −1354 to −1345 | In vitro binding assay with fzip homeodomain. |
| Chicken | HBS 2            | ETS1                                                | CTGAGTCAT       | −1369 to −1360 | Reporter activation in chicken embryo fibroblasts; deletion of response element. |
| Mouse | POU3F2 (BRN2)    | octamer                                             | ATGCAATG        | −201 to −193 | Reporter activation in mouse mouse N2A cells, but not in rat C6 cells. Binding assays (EMSA, footprint). |
| Mouse | EGR1 (KROX-24)   | EGR1                                                | TGGCGGCGCGCCT   | −187 to −165 | Inactivation of reporter activity by mutation of response element in N2A, C6 and NIH3T3 cells. |
| Mouse | PRX1 (PRX1)      | HBS                                                 | CATTAC          | −57 to −52 | Reporter activation in rat A10 vascular SMCs and RBL-6 lung fibroblasts, EMSA and supershift. |
| Mouse | MKL1             | SRE (CArg)                                          | CTATTATGG       | −1414 to −1423 | SRF-dependent reporter activation in NIH3T3 cells and inactivation by mutation of response elements. |
| Mouse | MKL1mutB1        | SRF-independent reporter activation by cyclic strain Chp |
| Mouse | MKL1             | MKL1mutB1                                           | CTATTATGG       | −247 to +147 | SRF-independent SAP domain dependent promoter activation in HC11 mammary epithelial cells |
| Human | SOX4             | MKL1mutB1                                           | CTATTATGG       | −247 to +147 | Transcripts induced in SOX4-transfected LNCaP cells. |
| Human | OTX2             | OT5                                                 | TAATCC          | −530 to −525 | 220bp promoter more active than longer constructs in SK-Mel-28 and InR1-G9 cells. Positive effect of first 20bp of first exon; inclusion of 1338bp of the first intron in the long promoter construct activates the reporter in SK-Mel-28, but not InR1-G9 cells. |
| Human | OTX2             | OT5                                                 | TCTAATCC        | −531 to −523 | In vitro binding assays. |
| Human | TGF-β and SMAD3 and 4 | ETS1, SMAD3 and 4                                   | TCTGG           | −66 to −62 | Reporter repression in U87-MG human glioblastoma cells; EMSA and binding studies. |
| Human | ETS1             | EB1                                                 | TCTGG           | −43 to −39 | Reporter activation in human dermal fibroblasts in a complex with CBP-300; mutation of response elements; DNA affinity precipitation; Co-IP’s of complexes. |
| Human | EB5              | EB5                                                 | TCTGG           | −121 to −118 | |
| Human | SP1              | EB5, SBS2                                           | TCTGG           | −39 to −36 | |
| Human | SBS2             | EB5, SBS2                                           | TCTGG           | −60 to −58 | |
| Human | SBS3             | EB5, SBS2                                           | TCTGG           | −39 to −37 | |
| Gene | Promoter species | Transcription factor overexpressed or factors added | Response element Sequence Position Functional assays |
|------|-----------------|-----------------------------------------------------|--------------------------------------------------|
| TNC  | Human 79        | PDGF and ETS1,2 and SP1 GGAAGGAT                    | TTCC -121 to -118 Reporter activation in human dermal fibroblasts after PDGF stimulation; mutation of response elements; overexpression of dominant negative TFs; Co-IP's of complexes |
|      | EBS1            | ETS1,2 and SP1 GGAA                                  | -76 to -68 |
|      | EBS3            | ETS1,2 and SP1 GGAA                                  | -39 to -36 |
|      | EBS4            | ETS1,2 and SP1 GGAA                                  | -60 to -58 |
|      | SBS2            | GGAAGGAT                                             | -39 to -37 |
|      | SBS3            | GGAAGGAT                                             | -60 to -58 |
|      | PDGF and FLI1   | GGAAGGAT                                             | -39 to -37 |
|      | SBS2            | GGAAGGAT                                             | -60 to -58 |
|      | SBS3            | GGAAGGAT                                             | -39 to -37 |
|      | Human 84        | EWS-FLI; EWS-ERG (EWS-ETS) EBS1-4                   | GGAA -130 to -30 Reporter activation in H1299 cells. Endogenous transcripts induced by EWS-ETS, but not FLI or ERG |
|      | Human 85        | JUN GCN4                                             | TGAGTG -149 to -144 Synergistic reporter activation; mutation of response elements EMSA; transcripts induced by Jun overexpression in rat embryo fibroblasts. |
|      | RELA (p65)      | RELA (p65)                                           | GGAATTCT -214 to -207 |
|      | NFkB            | RELA (p65)                                           | -79 to -73 |
|      | Human 92        | NOTCH2 RBPJk                                         | GTGGGAA -467 to -462 Reporter repression in human dermal fibroblasts (both basal and IL-4 or TGF-β induced); mutation of binding element; ChIP |
|      | Human 83        | GATA6                                                 | TATCC -214 to -207 Deletion of binding element inhibits promoter activation; EMSA; Overexpression of dominant negative regulators of NFκB inhibit strain-induced promoter activation in neonatal rat cardiac myocytes. |
|      | Human 92        | NFκBIA (IkBα); IKBKB (IKK-β)                         | GGAATTCCCT -214 to -207 |
|      | NFκB            | GGAATTCCCT                                           | -214 to -207 |
|      | Human 92        | NFκBIA (IkBα); IKBKB (IKK-β)                         | -167 to +435 167bp promoter was sufficient for full activity of reporter expression in cell lines of neural or glial origin but not in NIH3T3 and P19 teratocarcinoma cells. |
|      | Human 99        | NFκBIA (IkBα); IKBKB (IKK-β)                         | -57 to +40 57bp promoter was sufficient for full activity of reporter expression in cell lines of neural or glial origin but not in SK-MEL28 and HeLa cells. |
| TNR  | Mouse 101       | GGAAGGAT                                             | -150 to +333 More active than longer or shorter constructs in mouse L and human 293T cells |
|      | Mouse 101       | SP1 GGGAGG                                           | -145 to -150 Mutation of binding element reduces reporter activity; EMSA and supershifts. |
|      | Human 124       | SP1 GGGAGG                                           | -181 to +88 Higher activity in HT1080 cells than longer or shorter constructs; TSS in HT1080 cells and fibroblasts at +46bp. |
| TNC  | Human 121       | SP1 SP3 GGG...GGG...GGG...GGG...CCC                 | -33 to -76 Activation of 311bp and 181bp reporter constructs in Drosophila S9 cells. Mutation of binding sites inhibits promoter activity; EMSA and supershifts. |
The Tnc promoter, which is conserved in the human and also the chicken gene. In addition, the proximal promoter of the chicken, mouse and human TNC gene contains another conserved homeodomain binding sequence (HBS). Paired-related homeobox 1 (PRRX1; formerly called PRX1 or Mhox), a transcription factor involved in limb and craniofacial morphogenesis, is a marker for periosteal cells and often co-expressed with TNC. Overexpression of PRRX1 strongly induced a full-length mouse Tnc promoter construct in a vascular smooth muscle cell line. Later, PRRX1 was demonstrated to trans-activate Tnc gene transcription in mouse pulmonary endothelial cells through direct interaction with the HBS located within the proximal promoter. Furthermore, increased deposition of TNC along the arterial wall in pulmonary vascular lesions of patients with mutated BMP type II receptors was highly associated with the expression of PRRX1.

TNC is a prominent early marker for developing tendons. The basic helix-loop-helix transcription factor scleraxis (SCX) is essential for development of load-bearing tendons. The Tnc gene was therefore assumed to be a target gene of this tendon-specific transcription factor. In Scx null mouse embryos, however, TNC still accumulates in condensing mesenchyme where tendons normally develop. Therefore, the Tnc gene appears to be controlled by factors that act upstream of SCX during early tendon morphogenesis, such as PRRX1 (see above).

Figure 2. Scheme of the gene promoters of the 4 tenascins. The transcription start sites (TSS) are indicated with blue arrows in front of the first exons (e1; blue boxes). The start codons (ATG) of the translation start sites are marked with red arrows in the second (e2) or third exon (e3; for TNR), respectively. The upstream promoter sequences are represented by horizontal light blue lines, on which the experimentally confirmed transcription factor binding sites are marked by vertical bars. Dark blue color refers to those sites reported for the human promoters; sites described so far in the mouse promoters only are labeled in green. For nomenclature of binding sites and transcription factors, see the list of abbreviations. For additional information and publications on individual binding sites, refer to Table 1. Note that the promoter sequences are not drawn to scale. However, the exact location of each binding site is indicated below the bars; numbers refer to the distance in base pairs from the transcription start site.
Tenascin-C gene regulation by growth factors during tissue repair

In the adult, TNC protein is restricted to few tissues. However, the protein becomes prominently expressed *de novo* in practically every tissue upon inflammation in response to chemical or mechanical injury, as well as due to other pathological processes. A number of cytokines has been shown to induce TNC expression in different cell types, among them pro-inflammatory (IL-1α, IL-1β) as well as anti-inflammatory (IL-4, IL-13). Cytokines signal via various intracellular pathways (JAK/STAT, MAPK, NF-κB). However, there are no direct functional studies so far on the control of TNC promoter activity by cytokines.

More is known about TNC gene regulation during wound repair. Transforming growth factor-β (TGF-β) plays an important role as inducer of extracellular matrix protein expression during development and in tissue regeneration. Stimulation of TNC synthesis has been detected after treatment of chicken embryo fibroblasts with TGF-β1. A direct role of TGF-β in promoting TNC expression was observed in mammary epithelial cells (HC11) and in mouse embryo fibroblasts. For astrocytes, it was shown that the expression of TNC is controlled by the canonical SMAD-mediated TGF-β signaling pathway and by fibroblast growth factor (FGF).

In the same cells, platelet-derived growth factor (PDGF) regulates TNC gene expression via PI3K/AKT signaling, which triggers the interaction of transcription factors SP1 and ETS1 in an active complex that recognizes ETS binding sites (EBS) in the promoter. In the promoter, SMAD2/3 binding sites in the proximal promoter region were identified. In any case, these examples show that depending on the cell type and on the exact mode and dosage of mechanical stress, the TNC gene appears to be regulated via distinct mechanotransduction pathways (Table 1).

Interestingly, a GAGAC/TC motif was identified in this region. This motif is present in the control regions of other mechanoresponsive genes where it is recognized by NFκB, but the factors binding to it in the chicken TNC promoter have not been identified. In any case, these examples show that depending on the cell type and on the exact mode and dosage of mechanical stress, the TNC gene appears to be regulated via distinct mechanotransduction pathways.
Glucocorticoids are potent anti-inflammatory steroid hormones. They function by binding to nuclear receptors that act as transcription factors, but can also negatively regulate gene expression by inhibiting the activity of other factors like AP1 and NFkB. Glucocorticoids have also been described as important hormones involved in myelopoiesis, and they can directly act at the progenitor cell level or by modifying the expression of ECM components. Down-regulation of TNC expression by glucocorticoids was shown in bone marrow stromal cells. These authors suggested that the different TNC distribution between bone marrow of newborn and adult mice controlled by glucocorticoids might in part influence the hematopoiesis process. Putative binding sites for glucocorticoid receptors have been identified in the chicken TNC promoter sequence, but their function in the hormone response has not been explored. A further repressor motif was mapped in the human TNC promoter and was demonstrated to bind GATA6, a zinc finger transcription factor known to regulate the synthetic phenotype of vascular smooth muscle cells. Exogenous expression of GATA6 in dermal fibroblasts negatively modulated the level of TNC protein, and inhibited its induction by IL-4 and TGF-β (Table 1).

Tenascin-C gene regulation in cancer

Ets binding sites within the TNC promoter are not only important for its activation by the PDGF/Pi3K/Akt pathway (see above), but were also shown to be the targets of EWS-ETS transcription factor. EWS-ETS is a chimeric gene found in several tumor types such as Ewing sarcoma and peripheral primitive neuroectodermal tumors. In a similar setting, oncogenic transformation of primary rat embryonic fibroblasts can be the consequence of the activity of transcription factor c-Jun in cooperation with an activated Ras gene. The transient expression of TNC induced by c-JUN could facilitate the de-adhesion of fibroblasts from the extracellular matrix, thus promoting their transformation. The c-JUN transcription factor contains a bipartite DNA binding domain which recognizes GCN4/AP1 and NFκB binding sequences, located in the human TNC promoter region (see Table 1 and Fig. 2).

SOX4 is a transcription factor overexpressed in many human tumors, and TNC was identified as a direct SOX4 target gene. TGF-β is also associated with the enrichment of TNC protein in the stroma of malignant breast tumors. Through gene set enrichment analysis it was found that direct target genes of TGF-β–activated SMAD3 were also enriched in the list of the SOX4 target genes. In the context of malignancies, this would suggest a cooperation between SOX4 and TGF-β1 in controlling TNC expression.

In addition to SOX4, other patterning genes might be involved in TNC accumulation during malignancy in a cell- and tissue-specific manner. For example, overexpression of POU3F2 (Brn2; see 2.2.) stimulated transcription from the Tnc promoter in a neuroblastoma cell line, but had no effect in glioma cells. Interestingly, PRRX1, shown to induce TNC in vascular smooth muscle cells (see 2.2.) was identified as an important inducer of mesenchymal-epithelial transition both in the embryo as well as in cancer cells. Thus, PRRX1 might also play a role in TNC induction in tumors to facilitate local invasion.

Notch is a large transmembrane protein that acts as receptor for cell-bound ligands Delta and Jagged; upon activation, its intracellular domain is cleaved and translocates to the nucleus where it acts as transcriptional regulator through binding to RBPjκ/CSL. High levels of Delta-like-1 and Jagged-1 ligands expressed in many glioma cell lines and primary human gliomas were shown to be important for the induction of the Notch signaling pathway. The proximal promoter of the human TNC gene includes an RBPjκ/CSL binding sequence responsible for Notch2-mediated trans-activation in glioblastomas, and is likely to mediate strong TNC induction in these tumors. Conversely, in lung metastases of breast cancer, TNC accumulation has been implicated in supporting the Notch signaling pathway. Taking the 2 findings together, there may be a positive feedback between Notch and TNC expression, which in turn will further amplify the Notch signaling pathway.

Posttranscriptional regulation of tenascin-C gene by microRNAs

The role of microRNAs as regulators of post-transcriptional gene silencing is well documented, and recent studies have shown that the repression of certain microRNAs corresponds to a more pronounced tumorigenesis. For example, downregulation of SOX4 and TNC is controlled by miR-335, and loss of this microRNA in breast cancer was shown to induce metastasis in part by increased TNC levels. Other findings show how TNC promotes oncosphere formation by a metastasis-initiating breast cancer cell population for lung colonization, and in this context, GATA3 and miR-335 were downregulated.

Tenascin-R: An ECM Protein Mainly Restricted to the Central Nervous System

Structure of the tenascin-R (TNR) gene

The human TNR gene (gene ID: 7143) is located on chromosome 1q24 and contains 23 exons. The transcript starts with 2 non-coding exons, separated by large introns from exon 3 which contains the ATG start codon (see 2.2.) (Fig. 1). The non-coding part of the tenascin-R gene spans a much larger region than the region of the protein encoding exons (Fig. 1). As demonstrated by S1 nuclease analysis, TNR transcripts of fetal, adult, and neoplastic human brain all contained both exons 1 and 2, indicating the presence of a single TSS at exon 1. The proximal promoter region is not recognizable as such and lacks a TATA box, CAAT box, GC-rich regions, or an initiator element, but potential binding sites for GATA1/2, MyoD, glucocorticoid receptor and homeobox binding sites were present in the region of −111 to −974bp. A non-typical TATA box, multiple GAGA boxes and an initiator-like element were identified within exon 1. However, inclusion of exon 1 did not play any role in the transcriptional activation directed by a 230 bp promoter construct and a short 57bp promoter construct was sufficient for full and cell type-specific activity of the human TNR promoter in cell culture (Table 1 and Fig. 2). The gene and promoter structure is highly conserved also in the rat and the mouse. The TATA-less
mouse TNR promoter displays canonical binding sites for potential regulators such as GATA-1/2, AP1 and p53 transcription factors as well as glucocorticoid receptors. However, all these binding sites are outside the 167bp short promoter region and exon 1 shown to be sufficient for the induction of transcription in cells of neuronal origin. Expression of tenascin-R in neural development, injury and cancer

TNR, originally designated as janusin in rodents and restrictin in chicken, is almost exclusively located to the central nervous system, but transiently appears also in Schwann cells during peripheral nerve development. Previous work has shown that 2 TNR splice variants of 160 and 180 kDa are expressed in the central nervous system by oligodendrocytes and a few neuronal cell types, but not by astrocytes or fibroblasts. In the developing human cortex, the spatiotemporal distribution of TNR parallels neuronal migration. Conversely, TNR can inhibit neurite outgrowth either by interacting with adhesion molecule contactin (F3/F11) or by interfering with integrin-dependent adhesion to fibroblasts.

In the injured visual pathway of the lizard that has the capacity to regenerate, TNR protein expression with the subsequent loss of its anti-adhesive properties. On the other hand, TNR is up-regulated in the injured visual pathway of the lizard that has the capacity to regenerate. In brain cancer, TNR was reported to be overexpressed in pilocytic astrocytoma, oligodendroglioma and ganglioglioma, but not glioblastoma.

Tenascin-R gene regulation by growth factors

In mice, oligodendrocyte precursor cells synthesize most tenascin-R, whereas expression decreases with differentiation. In more mature oligodendrocytes, expression of TNR was stimulated by coculture with astrocytes or neurons, and was also induced by adding platelet-derived growth factor (PDGF) but not basic fibroblast growth factor. Rat pheochromocytoma cells (PC12) express high levels of Tnr mRNA after nerve growth factor (NGF) treatment. In contrast, rat oligodendrocytes treated with conditioned medium from activated microglia show a reduced Tnr mRNA expression due to the release of injury factors such as TNF-α. Unfortunately, there are no studies yet how these growth factors and cytokines regulate the TNR gene on the promoter level.

Tenascin-X: A Regulatory Component of Collagen Fibers

Structure of the tenascin-X (TNXB) gene

The human TNX gene was discovered as unknown “gene X” present in the major histocompatibility locus III (MHCIII). There, it is found on the opposite strand of the CYP21A2 gene and partially overlaps with it. CYP21A2 encodes a steroid 21-hydroxylase, and mutations in this gene cause congenital adrenal hyperplasia. However, a fraction of cases with deletions in this genomic region are also deficient for TNX; these patients suffer in addition from hyperextensible skin and joint laxity typical of Ehlers-Danlos syndrome. The MHCIII gene locus is very complex and has been partly duplicated resulting in 2 TNX gene copies TNXA and TNXB, of which TNXA is a truncated version.

Expression of tenascin-X in collagen-rich tissues

As mentioned above, mutations or deletions in the human TNXB gene cause Ehlers Danlos syndrome, and certain TNXB mutations are reported to cause another connective tissue disorder, vesicouretral reflux. Deletion of the Tnxb gene in mice was subsequently shown to phenocopy the connective tissue defects observed in affected human patients. Thus, in apparent contrast to the other tenascins, TNX protein has a clear structural role in connective tissue integrity. It is reported to (indirectly) bind to and bridge collagen fibrils and regulates collagen deposition in vivo. During rat embryonic development, Tnxb mRNA is especially prominent in the epicardium, skeletal muscle connective tissue, and tendon primordia. In the adult pig, TNXb mRNA becomes widely and constitutively expressed in most connective tissues, but is present at higher levels in tendons, ligaments, and perineural sheaths. Despite of considerable overlap on the tissue level especially in the embryo, on a smaller scale the distribution of mouse Tnxb mRNA and TNX protein was found to be distinct and often reciprocal to that of TNC.
no report indicating an induction of tenasin-X expression in inflammation or wound healing.

**Tenascin-X gene regulation by growth factors and hormones**

Unlike for the other proteins belonging to the tenasin family, there are so far no reports indicating that TNX is regulated by growth factors or cytokines. Thus, the signaling pathways that act on the Sp1/Sp3 sites described above in the TNX promoter are at present unknown. Like TNC and TNW, however, TNX is subjected to negative regulation by glucocorticoids, but again the mechanism has not been elucidated yet on the gene promoter level.

**Tenascin-X gene regulation in cancer**

Not only during embryogenesis, but also in the context of malignancy TNX appears to be regulated in an opposite way compared to TNC. For example, TNX expression is prominent in normal pig skin but strongly suppressed in cutaneous melanoma, where TNC is highly upregulated. In contrast to TNC, TNX is not induced in breast and ovarian carcinomas, but has been reported to be a marker for malignant mesothelioma.

**Tenascin-W: Expression in Osteogenesis and Tumorigenesis**

**Structure of the tenasin-W (TNN) gene**

The first tenasin-W gene to be discovered and cloned was from zebrafish and named tnn. Unfortunatly, its mouse ortholog was subsequently called tenasin-N; Tnn for the mouse and TNN for the human are now the official gene names in the NCBI data base. The complete characterization of the human TNN gene (ID: 63923; chromosome 1p23-q24) was carried out in 2007. The human TNN gene consists of a total of 19 exons spanning 80 kb of genomic DNA and the transcript starts with a non-coding exon (Fig. 1). The same gene organization is found for mouse Tnn, except for presence of 3 additional exons encoding 3 additional fibronectin type III repeats. Thus the mouse TNW protein is about 30kDa larger than the human counterpart. The first non-coding exons as well as about 600bp 5' of the transcription start are highly conserved between human and mouse orthologs indicating the presence of conserved promoters, as revealed by the conservation tracks of the UCSC genome browser (http://genome.ucsc.edu/). Using ConSite (http://consite.genereg.net/) to explore transcription factor binding sites shared by the putative human and mouse promoter sequences revealed a conserved TATA box as well as conserved SMAD binding elements. However, the relevance of these sites and the functionality of the TNN promoter still need to be tested experimentally.

**Expression of tenasin-W in bone formation and cancer**

TNC and TNW proteins show partially overlapping expression patterns in the developing and adult skeleton. Most of the research based on the regulation of TNW expression in a physiological context indicates its significant role in osteogenesis. For instance, addition of TNW to explant cultures of frontal bones increased their growth, suggesting that TNW can accelerate bone formation in a complex multicellular environment. Furthermore, in a transgenic mouse model, overexpression of GFP under a Prx1 promoter was used to isolate osteochondro-progenitor cells from the periosteum. These cells expressed high levels of TNW protein, proving its association with PRRX1-positive bone progenitor cells. Thus, it is tempting to speculate that similarly to TNC, TNW might be regulated by PRRX1. In the adult organisms, TNW is predominantly expressed in the periosteum as well as in other stem cell niches similar to TNC.

In tumors, TNW is again similarly distributed as TNC and high amounts are present in cancer stroma of most solid tumors. However, in comparison to TNC, TNW represents an even more specific tumor marker in several malignancies such as in kidney, lung and colon cancers. In brain cancers TNW was found to be specifically expressed in blood vessels, and in vitro studies showed pro-angiogenic activity of TNW added to endothelial cell cultures. Also in kidney and lung cancer, a strong association of TNW expression was found with tumor blood vessels. These studies support the potential of TNW as a tumor biomarker. Its strict association with blood vessels suggests a good accessibility from the blood stream for antibody-drug-conjugate based therapeutic strategies.

**Conclusions and Outlook**

The four members of the vertebrate tenasin family are quite similar not only in their overall domain organization, but as typical "matricellular" proteins also appear to fulfill similar functions: There is increasing evidence that they all modulate cell adhesion and cellular responses to growth factors and cytokines in a context-dependent manner. In view of these structural and functional similarities, it is surprising that the 4 tenascins exhibit
vastly different expression patterns in space and time. TNR is almost exclusively found in the central nervous system and its expression level is affected by just a few known growth factors. In contrast, TNC is an “oncofetal” or “stress” protein that is controlled by many stimuli and can appear in almost any tissue and cell type in the embryo and the adult, however just at certain times and in specific locations. TNW is a constitutive component of most connective tissues and its level is barely influenced by growth factors, whereas the expression of TNW is again more similar to that of TNC, although it is even more restricted to developing/remodeling bone, certain stem cell niches, and to a subset of tumors. These observations point to very distinct mechanisms of regulation for the various family members. Fitting with a highly regulated versus a more constitutive expression, respectively, the gene promoters of \textit{TNC} and \textit{TNW} have classical TATA boxes ca. 20–40 bp upstream of their transcription start sites, whereas the promoters of \textit{TNR} and \textit{TNXB} are TATA-less. \textit{TNC} and \textit{TNR} genes have a first untranscribed exon separated from the second ATG-containing exon by a very large intron, which is likely to be involved in gene regulation. \textit{TNR} has 2 untranscribed exons and the ATG translation start site is found in the third, whereas \textit{TNXB} even possesses 3 alternative promoters and non-coding first exons that are subjected to alternative splicing (see above).

Because the \textit{TNC} gene was the first of the family to be characterized, most is known about its regulation, although it turns out to be overwhelmingly complex. The responsiveness of the \textit{TNC} gene to segmentation genes, growth factors/cytokines and mechanical stress appears to be very similar in different vertebrate species, which is reflected in the high sequence similarity in parts of the promoter regions. Nevertheless, although many of the same cis-acting regulatory elements have been identified in the chicken, mouse and human \textit{TNC} promoter, there appear to be differences in their arrangement and activity. Although TNW is quite distantly related to TNC within this protein family, its expression pattern and the regulation of its gene \textit{TNW} appears to be most similar to that of \textit{TNC}, especially also in stem cell niches and in cancer. Future research is likely to reveal more about similarities and differences in the control of these 2 genes e.g. by TGF-\textbeta\text{s} vs. BMPs, or by various cytokines. Conversely, TNR is the closest family member of \textit{TNC} on the protein level, but it is regulated completely differently. It is remarkable that only about 200 bp of the proximal promoter and sequences in the first exon of the \textit{TNR} gene are necessary and sufficient for its expression exclusively in neuronal cells. Thus, this gene appears to represent a relatively simple case of tissue-specific regulation, and it will be interesting to work out the exact mechanism. As for \textit{TNX}, it exhibits a constitutive expression more like \textit{TNR}, but in a reciprocal fashion since it is found in most tissues except the CNS. Nothing is known yet about the mechanism for tissue-specific expression of the \textit{TNXB} gene, and the lack of regulation by growth factors and the relative scarcity of putative cis-acting elements in its promoter are noteworthy. For more meaningful comparisons between the genes of this family, it would be important to learn more about the regulation of \textit{TNR}, \textit{TNXB} and \textit{TNWN} genes in the future. In the case of \textit{TNC}, systems biology and computational approaches will probably be required to fully understand how a dozen or more signaling pathways converge to control its very complex gene promoter.

Why is it relevant to study the regulation of tenascins in even more detail? \textit{TNR} and \textit{TNX}, because of their largely constitutive expression, might perhaps be less interesting in this respect. Of course, \textit{TNX} will remain in focus because of its important function in tissue integrity and its association with human disease, and \textit{TNR} might be further investigated as a prime example for highly tissue and cell type-specific gene regulation. In case of the highly regulated \textit{TNC} and \textit{TNW}, however, more and more evidence suggests that these 2 proteins are important modulators of cell division, migration and differentiation in adult stem cell niches as well as in cancer. Moreover, because of their very localized and high expression in the extracellular matrix, both \textit{TNC} and \textit{TNR} are very well suited for targeting antibodies and drugs to certain types of tumors. For this therapeutic approach to work effectively, it is important to know how \textit{TNC} or \textit{TNW} gene expression is affected e.g., by cytotoxic drugs in combined therapy, and what signaling pathways are involved. From a basic research point of view, the tenascins provide an intriguing example for a vertebrate protein family of paralogs with similar structure and function, but with distinct expression patterns in space and time that are generated by different mechanisms of regulation of the respective genes.

\textbf{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

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