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REVIEWS

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The Tenascin Family of ECM Glycoproteins: Structure, Function, and Regulation During Embryonic Development and Tissue Remodeling

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ABSTRACT **The determination of animal form depends on the coordination of events that lead to the morphological patterning of cells. This epigenetic view of development suggests that embryonic structures arise as a consequence of environmental influences acting on the properties of cells, rather than an unfolding of a completely genetically specified and preexisting invisible pattern. Specialized cells of developing multicellular organisms are surrounded by a complex extracellular matrix (ECM), comprised largely of different collagens, proteoglycans, and glycoproteins. This ECM is a substrate for tissue morphogenesis, lends support and flexibility to mature tissues, and acts as an epigenetic informational entity in the sense that it transduces and integrates intracellular signals via distinct cell surface receptors. Consequently, ECM-receptor interactions have a profound influence on major cellular programs including growth, differentiation, migration, and survival. In contrast to many other ECM proteins, the tenascin (TN) family of glycoproteins (TN-C, TN-R, TN-W, TN-X, and TN-Y) display highly restricted and dynamic patterns of expression in the embryo, particularly during neural development, skeletogenesis, and vasculogenesis. These molecules are reexpressed in the adult during normal processes such as wound healing, nerve regeneration, and tissue involution, and in pathological states including vascular disease, tumorigenesis, and metastasis. In concert with a multitude of associated ECM proteins and cell surface receptors that include members of the integrin family, TN proteins impart contrary cellular functions, depending on their mode of presentation (i.e., soluble or substrate-bound) and the cell types and differentiation states of the target tissues. Expression of tenascins is regulated by a variety of growth factors, cytokines, vasoactive peptides, ECM proteins, and biomechanical factors. The signals generated by these factors converge on particular combinations of *cis*-regulatory elements within the recently identified TN gene promoters via specific transcriptional activators or repressors. Additional complexity in regulating TN gene expression is achieved through alternative splicing, resulting in variants of TN polypeptides that exhibit different combinations of functional protein domains. In this review, we discuss some of the recent advances in TN biology that provide insights into the complex way in which the ECM is regulated and how it functions to regulate tissue morphogenesis and gene expression.** ***Dev Dyn 2000;218:235–259.***

Key words: extracellular matrix molecules; oranogenesis; cell adhesion; homeobox transcription factors; matrix metalloproteinases; integrins; biomechanical factors

INTRODUCTION: IDENTIFICATION, STRUCTURE, AND VARIATION OF TENASCIN POLYPEPTIDES

The first member of the tenascin family, tenascin-cytotactin (TN-C), was discovered independently by several laboratories investigating different aspects of cell, developmental, and tumor biology. Accordingly, it was given a number of names including glial/mesenchymal extracellular matrix protein (GMEM) (Bourdon, 1983), myotendinous antigen (Chiquet and Fam-

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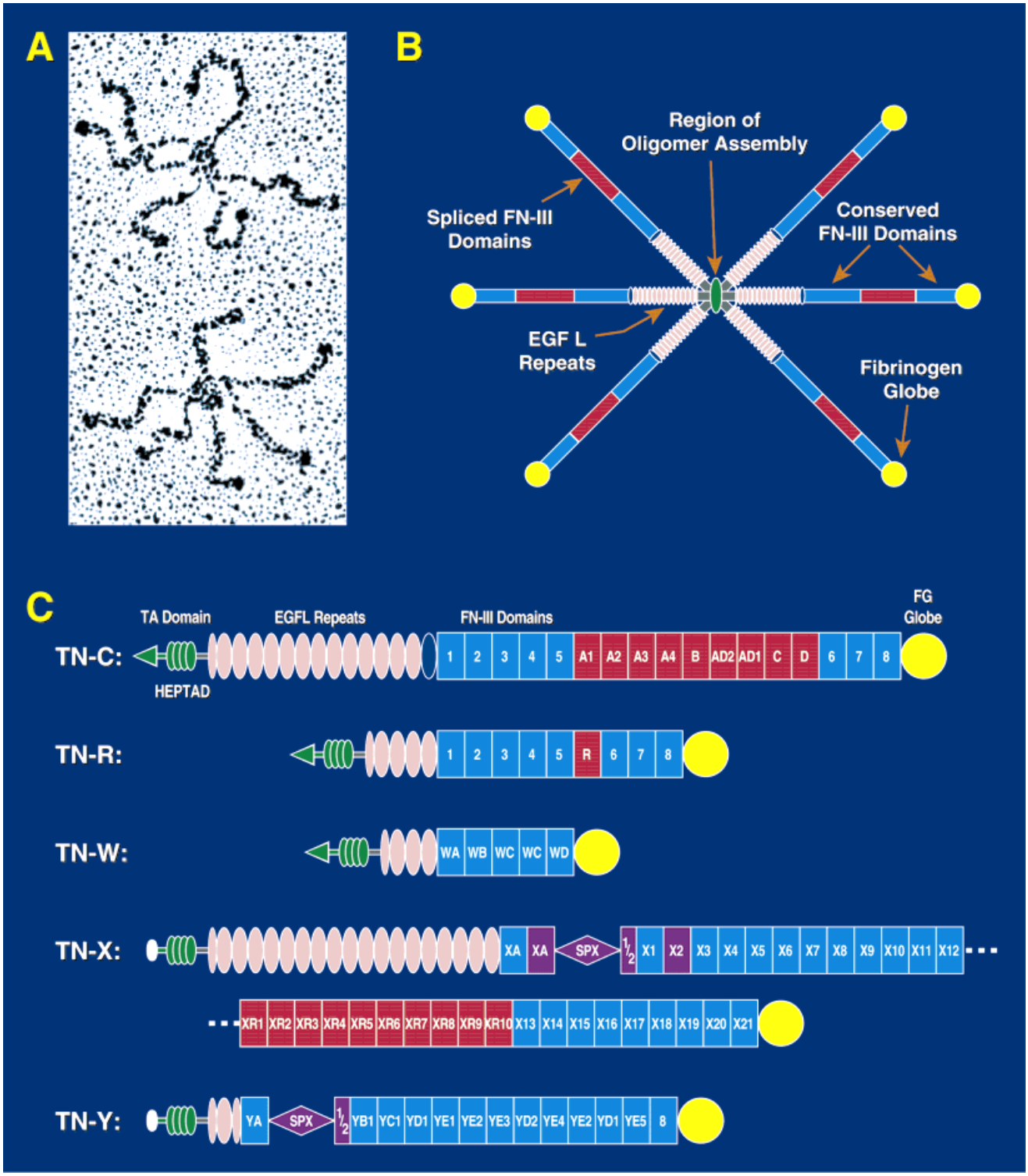

Figure 1.

brough, 1984a; Chiquet and Fambrough, 1984b), hexabrachion (Erickson and Iglesias, 1984), cytotactin (Grumet et al., 1985), $J1_{220/200}$ (Kruse et al., 1985), tenascin (Chiquet-Ehrismann, 1986), and neuronectin (Rettig et al., 1989). TN-C plays a morphoregulatory role during development, tissue remodeling, and in disease by regulating the cell adhesive and signaling properties of neural and non-neural cells. Because most of the

functional and regulatory studies have concerned TN-C, it will be the major focus of this review.

Four other proteins (TN-R, TN-W, TN-X, and TN-Y) have the same consecutive arrangement of domains that are found in TN-C (Fig. 1C). TN-R, also known as restrictin, janusin, and $J1_{160/180}$ (Fuss et al., 1991; Rathjen et al., 1991; Norenberg et al., 1992), is synthesized by oligodendrocytes during myelination in late gestation and early postnatal development, and its expression partially overlaps with that of TN-C. TN-W was identified in the zebrafish and is expressed prominently in neural crest pathways and colocalizes with TN-C in several developmental contexts (Weber et al., 1998). TN-X is part of a large gene array on human chromosome 6 that includes the MHC complex and genes encoding steroid 21-hydroxylase, complement C4, and a CREB-like transcription factor (Morel et al., 1989; Bristow et al., 1993). Several recombination events occur in this gene locus, resulting in immune deficiencies, adrenal hyperplasia, and juvenile rheumatoid arthritis (Jaatinen et al., 1999; Rupert et al., 1999; Yang et al., 1999). TN-X was independently isolated as flexilin, a bovine protein that is a prominent component of connective tissues (Lethias et al., 1996; Elefteriou et al., 1997). Consistent with an essential role for TN-X in development, deletions within the TN-X gene results in Ehlers-Danlos Syndrome, a connective tissue disorder involving skin and joint hyperextensibility, vascular fragility, and poor wound healing (Burch et al., 1997). TN-Y is a protein that is expressed in connective tissues and in the brain that modulates muscle cell growth (Hagios et al., 1996; Hagios et al., 1999; Tucker et al., 1999).

Overall Structure of Tenascin Polypeptides

TN-C is a disulfide-linked oligomer having subunits that range between 190 and 300 kDa and is visualized in rotary shadowing images as a striking, highly symmetrical structure called a hexabrachion (Erickson and Iglesias, 1984) (Fig. 1A). In the TN-C hexamer, six arms emanate from a central core, the proximal portions of the arms are thin and rigid, the distal portions are thick and flexible, and the ends of the arms contain an electron dense globular particle. Characterization of proteolytic fragments and cDNA clones of TN-C allowed the formulation of a detailed model for TN-C (Jones et al., 1989; Spring et al., 1989). In the TN-C hexabrachion, six polypeptide chains are linked at their amino termini via a tenascin assembly (TA) domain, the thin portions of the arms are composed of a linear array of epidermal growth factor-like (EGFL) repeats, the thick portions of the arms are composed of a series of fibronectin type III (FN-III) domains, and the terminal knob is composed of a globular domain that resembles the carboxyl terminal portion of the β and γ chains of fibrinogen (see Fig. 1B). The other four members of the tenascin family contain the same linear arrangement of the four protein domains that are found in TN-C (Fig. 1C). Some overall structural properties of tenascin (TN) domains are provided below. A more detailed description of the features of individual domains is presented in the legend of Figure 1.

Tenascin Assembly (TA) Domain

The TA domain contains cysteine residues and between 3 and 4 α-helical heptad repeats (Conway and Parry, 1991) that enable the amino termini of TN polypeptides to be linked into oligomeric structures. These repeats are found in all known members of the TN family (Fig. 1C). Assembly of the TN-C hexabrachion is a two-step process, involving formation of trimers and then linkage of trimers into hexamers. Trimer formation is initiated by association of three TN-C polypeptides in a triple-stranded coiled coil by the hep-

Fig. 1. **A:** Rotary shadowing images of two mouse TN-C hexabrachions. Each arm is approximately 100 nm in length. **B:** Model of the TN-C hexabrachion. The tenascin assembly (TA) domain links six TN-C chains via the heptad repeats (green central hub). Outside of the heptad region is a series of cysteine residues with a highly conserved spacing that appears in all known TN polypeptides (gray rectangular portion of the arms). Proceeding in a carboxyl terminal direction, the domains are as follows: an array of EGFL repeats (salmon-colored ovals), two types of FN-III domains: (1) those conserved in all variants of TN-C (light blue rectangles) and (2) those that are alternatively spliced (red rectangles), and the terminal fibrinogen globe (yellow circle). **C:** Schematic diagrams of the five known tenascins. TN-C has 13 EGFL repeats that are common to all vertebrate species (salmon-colored ovals) and a 14th EGFL repeat that is found in mammalian species (navy blue oval). Otherwise, the FN-III and fibinogen domains have the same color coding as those found in (B). TN-R has 4.5 EGFL repeats and either 8 or 9 FN-III repeats. The extra FN-III domain (red rectangle designated R) is spliced at a position that is comparable with TN-C. Variation in TN-R polypeptides also occurs in the TA domain (Norenberg et al., 1992). TN-W contains five FN-III repeats (WA-WD) that are most similar to each other. TN-C, TN-R, and TN-W all contain amino terminal cysteine residues (represented by green triangles) that may allow all of these molecules to form hexamers. TN-X and TN-Y appear to lack the amino terminal cysteines required to stabilize the linkage of trimers into higher order oligomers. This lack of cysteines is indicated by a domain represented by a solid white oval. TN-X contains 18.5 EGFL repeats and at least 33 FN-III repeats. FN-III repeats in TN-X fall into three different categories: (1) those related to one another and that have not been shown to undergo alternative splicing (repeats X1-21), (2) those near the amino-terminal end of the array that are more related to one another (repeats designated XA), and (3) those that are alternatively spliced (see Ikuta et al., 1998), highly similar to one another, and probably arose from a recent duplication event (repeats XR1-XR10). TN-X also contains an unusual serine-proline rich (SPX) domain that was originally found in TN-Y (Hagios et al., 1996). Comparison of human, mouse, and bovine TN-X sequences reveal that at least one of the XA repeats, the exon encoding SPX and 1/2 of an FN-III domain, and the X2 repeat (corresponding to repeat M3 in the mouse TN-X gene) can be alternatively spliced (these regions are indicated in purple). TN-Y contains a heptad repeat, a small EGFL array containing one half, 1 complete, and another half of an EGFL repeat, an SPX-1/2FN-III domain similar to that in TN-X, 12 FN-III repeats, and a fibrinogen globe. Eleven of the 12 FN-III repeats in TN-Y (designated YA-YE) are significantly more related to those in TN-Y than to FN-III domains in the other tenascins. However, the last repeat is similar to repeat 8 from TN-C and TN-R.

tad repeats. Trimers are further stabilized by interchain disulfide bonds at two cysteine residues amino terminal to the heptad region (Kammerer et al., 1998). The clustering of TN-C chains creates a multivalent TA domain that interacts homophilically with another trimer to form a hexamer. Hexamers are then stabilized by disulfide linkages at a cysteine residue that is located 50 residues amino terminal to the heptad repeat.

TN-C is currently the only member of the TN family known to form hexabrachions. However, the additional cysteine residue that facilitates hexamer formation is also found in TN-R and TN-W. TN-R forms trimers (Norenberg et al., 1992), and because all known tenascins contain the heptad repeats, they are all likely to form trimers.

TN-C is also capable of forming nonamers (Schenk and Chiquet-Ehrishmann, 1994), indicating that native TN-C molecules exist predominantly as multiples of three. Heterotypic hexamers have been observed in which a trimer containing a particular variant of TN-C is linked to a trimer containing a different variant (Fischer et al., 1995; Fischer et al., 1997; Kammerer et al., 1998). Overall, these observations suggest that two types of heterotypic trimers (one made up of more than one splice variant of TN-C and another composed of more than one tenascin family member, e.g., TN-C and TN-R) might be possible. Assembly of heterotypic TN multimers might provide additional combinatorial diversity of ECM structure and function in cellular contexts in which more than one TN gene or splice variant is expressed. It is uncertain how prevalent TN oligomers are in the ECM and whether binding to particular ligands influences the ability of TNs to form such oligomers.

EGFL Array

The EGFL repeats in the tenascins (Fig. 1C) are 31 amino acids in length and contain six cysteine residues that participate in intrachain disulfide bonds. Tenascin EGFL domains form an exceptionally compact structure compared with that of epidermal growth factor (Cooke, 1987). Unlike EGFL repeats found in coagulation factors IX and X (Handford, 1990) and in the Notch, Delta, and Serrate receptors (Klein and Arias, 1998; Lendahl, 1998; Bishop et al., 1999), those of the TN family lack the acidic residues required for binding of calcium. Tenascin-like EGFL arrays are encoded by a single exon. To date, a true tenascin containing the consecutive arrangement of EGFL, FN-III, and FG domains found in vertebrates has not been identified in an invertebrate species. However, proteins containing TN-like EGFL domains have been identified in *Drosophila* in an extracellular protein called Ten-a (Baumgartner and Chiquet-Ehrismann, 1993), and in the transmembrane receptor protein Ten-m/Odz (Baumgartner et al., 1994; Levine et al., 1994). A Ten-m-related protein called teneurin-1 has also been found in vertebrates (Minet et al., 1999), and other vertebrate counterparts of Ten-m/Odz that are prominently expressed on axonal processes and delineate compartments during the development of the forebrain and midbrain have been identified (Mieda et al., 1999; Oohashi et al., 1999). Collectively, these and other studies (see Table 1) indicate that EGFL domains play key roles in neuronal migration and axon pathfinding during development.

Fibronectin Type III (FN-III) Domains

A large portion of TN polypeptides is made up of a series of FN-III domains (Fig. 1C). FN-III domains contain approximately 90 amino acids and are extended globular structures composed of seven antiparallel β-strands arranged in two sheets (Patthy, 1990; Baron et al., 1992; Leahy et al., 1992). FN-III arrays are highly elastic (Carr et al., 1997; Akke et al., 1998) and can be stretched to several times their length and refolded rapidly (Oberhauser et al., 1998). Particular peptide motifs within individual FN-III domains bind to several ligands including other ECM proteins, glycosaminoglycans, and a number of different cell surface receptors (see Table I). FN-III domains are susceptible to proteolytic degradation, allowing TN-containing matrices to be selectively remodeled, particularly by matrix metalloproteinases (MMPs) and serine proteases (Imai et al., 1994; Siri et al., 1995; Gundersen et al., 1997).

The nature and number of FN-III domains in tenascin polypeptides are altered by alternative RNA splicing. This can generate a considerable diversity in the functions of TN polypeptides (see Table I). In TN-C, at least nine different FN-III domains have been identified that are differentially included or excluded by RNA splicing (Jones et al., 1988; Jones et al., 1989; Spring et al., 1989; Gulcher et al., 1989,1991; Nishi et al., 1991; Weller et al., 1991; Sriramarao and Bourdon, 1993; Dorries and Schachner, 1994; Derr et al., 1997). Some of the TN-C splice variants occur at distinct frequencies during development of the nervous system, in smooth muscle, kidney, and the cornea (Jones et al., 1989; Prieto et al., 1990; Chiquet-Ehrismann, 1991; Kaplony et al., 1991; Tucker, 1991; Weller et al., 1991). Using RT-PCR, as many as 27 different FN-III variants of TN-C have been found during mouse brain development, indicating that many of the theoretically possible combinations of FN-III repeats are likely to be expressed during tissue morphogenesis (Joester and Faissner, 1999). Selection of particular TN-C splice variants is modulated by the proliferative state of the cell (Borsi et al., 1994), by extracellular pH (Borsi et al., 1995), and by polypeptide growth factors such as TGF-β1 (Zhao and Young, 1995). Currently, however, the mechanisms that determine preferential splice site selection of TN mRNAs in the region of the FN-III repeats are unknown. Alternative splicing of FN-III repeats occurs in two other members of the tenascin family, TN-R (Norenberg et al., 1992) and TN-X (Ikuta et al., 1998) (see Fig. 1C).

Fibrinogen Globe

The fibrinogen globe (Fig. 1C) contains polypeptide loops formed by two consecutive intrachain disulfide bonds (Doolittle, 1984). Comparisons with similar structures in the parvalbumin family of proteins indicate that the region of the fibrinogen globe between the intrachain disulfide loops contains an EF-hand calcium binding site (Tuffy and Kretsinger, 1975). Indeed, the fibrinogen domain in TN-C binds Ca^{2+} (Jones et al., 1989; Spring et al., 1989). Moreover, the calcium-binding property of the fibrinogen globe influences interactions with other proteins (see Table 1). Proteins having fibrinogen globes have been identified in invertebrate organisms, including FRePA, a molecule from the sea cucumber *Parastichopus parvimensus* (Xu and Doolittle, 1990), and the product of the scabrous gene from *Drosophila*, which is known to function in eye development (Baker et al., 1990). Evolutionary studies also suggest that proteins containing fibrinogen globes played developmental roles long before the emergence of fibrinogen-based blood clotting and the vertebrate lineage (Xu and Doolittle, 1990; Jones, 1991).

FUNCTIONS OF TENASCINS: INTERACTIONS OF DOMAINS WITH LIGANDS AND EFFECTS ON CELLS

Tenascins contain adhesive and counteradhesive activities that coexist on the native molecule. These opposing activities arise as a consequence of tenascins binding to other components of the ECM and to cell surface receptors. The tenascins bind with high affinities to several proteins and carbohydrates. Interactions of particular domains of tenascins (particularly, TN-C and TN-R) with these ligands and some of the cellular activities that these binding events evoke are presented in Table 1.

Cell surface receptors for the tenascins include members of the integrin family of heterodimers, cell adhesion molecules (CAMs) of the immunoglobulin superfamily, an unusual transmembrane chondroitin sulfate proteoglycan/receptor protein tyrosine phosphatase called phosphacan/RPTP ζ/β, and annexin II. TN-C and TN-R also interact with other extracellular proteins, including fibronectin, and also bind with high affinity to a class of extracellular chondroitin sulfate proteoglycans called the lecticans (aggrecan, versican, brevican, and neurocan). Furthermore, tenascins interact with and are cleaved by serine proteinases and matrix metalloproteinases.

The functional activities provided by tenascin proteins may occur through direct interactions with cell surface receptors or indirectly through modulation of other ECM proteins and cell-cell adhesion molecules. Although analysis of tenascin fragments has provided a convenient way in which to characterize ligand-binding sites, cell surface receptors, and associated intracellular pathways, these results must be interpreted with care because they may not accurately recapitulate activities ascribed to the native molecule.

Differential expression of one or more alternatively spliced forms of TN-C is one way to select for binding of particular cell types via preferential interactions with cellular receptors or ECM components. For instance, the alternatively spliced A–D region has also been shown to bind a particular cell surface receptor, annexin II (Chung and Erickson, 1994). In addition, the 190-kDa form of TN-C, in which variable FN-III domains are spliced out, binds to F11/contactin via an uninterrupted segment of the fifth and sixth type III domains (see Table I). This smallest TN-C variant also binds to fibronectin with higher affinity than larger forms and is the only variant that is expressed in tissues rich in extracellular matrix (Chiquet-Ehrismann et al., 1991). Larger variants of TN-C have been correlated with cell migration and invasiveness of carcinomas (Prieto et al., 1990; Kaplony et al., 1991).

Roles of Tenascins During Neural Development

One significant function of the tenascins during neural development is their ability to modulate homophilic and heterophilic binding between families of CAMs. The concept of the tenascins acting as a modulator or cross-linker between families of CAMs applies particularly well to TN functions in neuronal process formation, extension, and guidance during neural development. In this regard, numerous regions of TN-C and TN-R that have complex and frequently contradictory effects on neurite outgrowth, migration, and pathfinding have been identified (see Table 1). Some important findings in this arena emphasize that tenascins act on two different classes of cell surface adhesion molecules (CAMs), those of the immunoglobulin superfamily (including F11/contactin, axonin-1, and neurofascin) and phosphacan/RPTPζ/β. These interactions are modulated by additional TN/ECM interactions with members of the lectican family of chondroitin sulfate proteoglycans (brevican, aggrecan, versican, and neurocan).

The EGFL domain primarily functions as counteradhesive domain, particularly during the processes of neuronal migration and pathfinding during CNS and PNS development where it inhibits attachment of neuronal processes to cellular receptors on other neurons and glia or the ECM produced by these cells (Prieto et al., 1992; Lochter and Schachner, 1993; Crossin, 1994). In TN-R, EGFL repeats interact with phosphacan and F11/contactin (see Table I). TN-R/contactin binding results in activation of F11/contactin-induced serine/threonine kinase activity (Xiao et al., 1997). These findings suggest that TN-R binding might influence the signaling by Ig-CAMs in neurons, and this could be critical for modulation of neurite extension and repulsion. A major challenge for future studies is to test this idea in vivo.

The FN-III repeats within the tenascins have been shown to bind to Ig domains from several CAMs. This was initially established by the seminal study by

TABLE 1. Interactions of TN Domains With Ligands and Activities

	Ligands/cells/activities	References
TN-C domains		
EGFL; FN 7-8	Counteradhesion; fibroblasts, neurons, glia	Spring, 1989; Prieto, 1992
EGFL	Neuronal outgrowth, neurite repulsion	Fischer, 1997; Gotz, 1997
TN-C; EGFL	Rounding of NIH3T3 cells, suppression of growth; reduced pHi	Crossin, 1991
FG; FN III	Cell attachment and spreading	Krushel, 1994
FN 1-3	Cerebellar neuron attachment	Gotz, 1997
FN 1-3; FN A-D	Suppression of milk protein synthesis in mammary epithelium, tissue involution	Jones, 1995
FN 1-5	Fibronectin; T lymphocyte inhibition of β1 integrin adhesion	Hauzenberger, 1999
FN 2-6	Integrins; cell attachment	Prieto, 1992
FN 3	$\alpha_V\beta_3$, $\alpha_2\beta_1$; endothelial cell elongation	Sriramarao, 1993
	$\alpha_V\beta_3$, $\alpha_V\beta_6$; glioma spreading	Prieto, 1993
	$\alpha_V\beta_3$, $\alpha_9\beta_1$, $\alpha_V\beta_6$; colon carcinoma spreading	Yokosaki, 1996
	$\alpha_x\beta_1$; neuron attachment, inhibition of growth	Probstmeier, 1999
	$\alpha_8\beta_1$; smooth muscle cell, astrocyte adhesion	Schnapp, 1995
	$\alpha_8\beta_1$; K562 cell adhesion	Denda, 1998
FN 3,5,6	$\alpha_x\beta_1$; glioma process extension	Giese, 1996; Phillips, 1998
FN 3-5 (190 kD)	Perlecan; CHO cells, incorporation of TN into fibronectin-rich matrices	Chung & Erickson, 1997
FN A-D	Attachment of uterine epithelia, reduced adhesion and implantation	Julian, 1994
	annexin II; corneal epithelia, proliferation, migration, wound healing	Matsuda, 1999
	annexin II; endothelia, loss of focal adhesions; increase in mitogenesis and migration	Chung & Erickson, 1994, Chung, 1996
	primary neurons, outgrowth and guidance	Gotz, 1997, Meiners, 1999
FN A3	MMP-2, MMP-3; fetal membranes, tissue remodeling	Bell, 1999
FN 4-5	Neurocan via the core protein; 17nM k_d binding, EDTA-inhibitable	Rauch, 1997
FN 3-5; 5	Heparin binding	Aukhil, 1993, Weber, 1995
FN 5-6 (190 kD)	F11/contactin; inhibition by heparan sulfate to TN	Zisch, 1992, Weber, 1996
FN-III	CTB proteoglycan; neuron attachment to TN-C is inhibited by intact CTB-PG	Hoffman & Edelman, 1987; Friedlander, 1988
FN 6-8 (190kD)	Fibronectin; incorporation of TN-C into matrix	Chiquet-Ehrismann, 1991; Chung, 1995
FN 7-8	TN-C modulates FN-dependent induction of c-fos, collagenase, stromelysin, and gelatinase expression in synovial fibroblasts	Tremble, 1994
FG	Heparin	Chiquet-Ehrismann, 1988
	Phosphacan/RPTPζ/β; 12nM k_d binding, inhibits neuronal adhesion	Aukhil, 1993
FG + distal FN-IIIs	Neurocan via the core protein; Ca++ dependent	Milev, 1994, 1997; Grumet, 1994
FG	Aortic endothelial cells bFGF-induced sprouting, elongation	Schenk, 1999
FG	HSPG-dependent induction of bone marrow mononuclear cell supports growth in integrin independent manner	Seiffert, 1998
FN 6-8; FN 1-5		
TN-C	Syndecan	Salmivirta, 1991
	Glipican	Vaughan, 1994
	TAG-1/axonin-1; 9nM k_d binding	Milev, 1996
	Phosphacan/ RPTPζ/β (transmembrane form) via Asp-linked oligosaccharides in carbonic anhydrase + FNIII domains of phosphacan	Barnea, 1994; Milev, 1997
TN-C	Heparin inhibits attachment of PNS neurons to TN-C	Wehler-Haller & Chiquet, 1993, Chiquet & Wehler-Haller, 1994
TN-C	$\alpha_v\beta_3$-dependent reorganization of F-actin cytoskeleton and cell spreading, clusters EGF receptors and primes cells to mitogenic effects of EGF	Jones & Rabinovitch, 1996; Jones, 1997b
TN-C	Induction of c-myc, ICE and apoptosis of mammary epithelium	Boudreau, 1996
TN-R domains		
TN-R	F11/contactin; modulates neuron/glial adhesion	Rathien, 1991
NH_2-EGFL	F11/contactin; polarization of neuronal morphology, repulsion of F11-expressing processes is abolished by S/T kinase inhibitor H7	Xiao, 1996, 1997
	Phosphacan; Ca++ dependent hippocampal neurite outgrowth is neutralized by TN-R	

TABLE 1. (Continued.)

	Ligands/cells/activities	References
EGFL + FN 6-8	Attachment and then repulsion of neural cells transfected with VGSCβ subunits regulates channel activity	Xiao, 1999
FN 2-3	F11/contactin (Ig domains 1-3); TN-R enhances F11-mediated neurite outgrowth	Brummendorf, 1989; Norenberg, 1995
FN 3-5; 4-5	Binds lectin domains of lecticans, binding is Ca++ dependent and independent of carbohydrates. K_d's: Brevican 1nM; Aggrecan 12nM; Versican 15nM; Neurocan 30nM	Aspberg, 1997
TN-R	Fibronectin; inhibition of FN-mediated adhesion of mesenchymal and neural cells	Pesheva, 1994
TN-R	MAG; inhibits MAG-mediated repulsion of neurites	Yang, 1999
TN-R	Neurocan and phosphocan; via the core proteins	Milev, 1998
soluble TN-R	Increases tectal cell attachment via F11 anddecreases neurite outgrowth, F11/NrCAM binding is blocked	Zacharias, 1999
TN-R	Neurofascin; TN-R competes for neurofascin/Nr-CAM binding; shifts binding preference of Neurofascin from Nr-CAM to axonin-1 and F11/contactin	Volkmer, 1998
Other significant intermolecular interactions that may bear on TN biology		
TN-X FNX 9-10	$\alpha_v\beta_3$; mediates bladder carcinoma attachment	Elefteriou, 1999
FG	$\alpha_x\beta_1$; osteosarcoma attachment, but not spreading	
TN-Y	Binds C2C12 myoblasts, supports their growth	Hagios, 1999
Fibulin	Lectin domain of 4 lecticans	Aspberg, 1999
Neurocan; Phosphacan	Bind amphoterin and HB-GAM via chondroitin sulfate side chains, presence of amphoterin increases phosphacan/F11 binding	Milev, 1998
Neurocan	NCAM; decreases NCAM homophilic binding	Retzler, 1996
Neurocan; $\alpha_5\beta_1$; $\alpha_v\beta_3$ integrins	Bind to L1	Oleszewski, 1999
Neurofascin	TAG-1/axonin-1 and F11/contactin	Volkmer, 1998
Ng-CAM	TAG-1/axonin-1 and F11/contactin	Sonderegger, 1998

Rathjen et al. (1991) in which TN-R was discovered by virtue of its binding of the amino terminal Ig domains of F11/contactin. Subsequent studies established that TN-C and TN-R bind to several immunoglobulin-like CAMs, including neurofascin, TAG-1/axonin-1, and F11/contactin and that these binding events modulate homophilic and heterophilic interactions between these Ig-CAMs. Ig-CAMs bind primarily to the FN-III domains within the central portion of the smallest TN-C splice variant (190-kDa form).

TN-C purified from embryonic chick brain contains covalently linked chondroitin sulfate side chains and the carbohydrate antigen HNK-1 (Hoffman et al., 1988). These carbohydrates influence binding to core proteins and carbohydrates that are present on other cell adhesion molecules. For instance, interaction of TN-C and TN-R with glycosaminoglycans present on phosphacan and the lecticans influences the extent of interaction of TN-C and TN-R with immunoglobulin-like CAMs (Weber et al., 1996; Volkmer et al., 1998). Other examples of modulation of CAM-binding specificity by tenascins include the observations that binding of TN-R to neurofascin can switch this CAM's heterophilic binding preference for Nr-CAM to contactin (Volkmer et al., 1998). In addition, interaction of TN-C and TN-R with neurocan and phosphacan are likely to influence homophilic binding properties of N-CAM and members of the L1 family, because these proteoglycans interfere with homophilic binding of these CAMs (see Table I, Other interactions). Such alterations in CAM-binding specificity by the tenascins are likely to influence local neuron pathfinding programs, such as those that occur when commissural axons migrate contralaterally across the floorplate (Dodd et al., 1988) or projection and connection of thalamocortical axons to their targets (Gotz et al., 1996; Gotz et al., 1997).

The tenascins recently were shown to bind to other Ig-containing molecules and affect their functions. For instance, TN-R modulates the functions of the myelin-associated glycoprotein (MAG) (Yang et al., 1999) and the β-subunits of voltage-gated sodium channels (Xiao et al., 1999). These interactions open up novel arenas to examine the mechanisms underlying TN-dependent modulation of processes such as myelination and neuronal activity.

Control of Tissue-Specific Gene Expression and Apoptosis in the Mammary Gland

During normal tissue development, reciprocal interactions between the epithelial parenchyma and mesenchymal stroma play inductive roles in a variety of embryogenetic events (Grobstein, 1967). This also holds true for the adult organism, where structural alterations in either the parenchyma or stroma perturb tissue homeostasis (Sakakura et al., 1979; Cunha et al., 1985). In addition to signals derived from diffusible factors and cell-cell interactions, the development and function of the embryonic and adult mammary gland

and its tumor-forming counterpart are highly dependent on normal and aberrant signals originating from the ECM, including TN-C (Sakakura, 1991).

An examination of TN-C expression during normal mammary gland development and tumor formation indicates that it is differentially regulated at each stage of development and oncogenesis. For example, TN-C appears in the dense embryonic mammary mesenchyme at 14 days postgestation, but this diminishes in the postnatal and adult stages (Chiquet-Ehrismann, 1986; Inaguma et al., 1988). Low amounts of TN-C are detected in the maturing normal mammary gland, mainly localizing to blood vessels and as a discrete layer surrounding the ducts and acini (Inaguma et al., 1988; Howeedy et al., 1990; Nicolo et al., 1990). During pregnancy however, increasing amounts of progestins, estrogen, and placental lactogen trigger the mammary epithelium to proliferate from a branching ductal system to form the alveolar morphology (Neville and Daniel, 1987). At this stage of development, the alveolar cells are capable of synthesizing milk proteins, including β-casein, and TN-C production is suppressed. After parturition, prolactin and growth hormones act in concert with glucocorticoids to induce lactation. This phenotype represents the end point of the fully differentiated state and is typified by continued suppression of TN-C, growth-related genes, synthesis of milk proteins by the epithelium, and their vectorial secretion into an alveolar lumen (Jones et al., 1993; Marti et al., 1994; Marti et al.,1999).

After weaning, matrix metalloproteinase-mediated destruction of the basement membrane initiates the process of involution, characterized by loss of milk production and massive apoptosis. Involution restores the mammary gland to a phenotype that is essentially identical to that seen in the virgin animal (Hurley, 1989; Talhouk et al., 1991; Talhouk et al., 1992), and is accompanied by continued synthesis of high levels of myoepithelial cell-derived TN-C that surrounds the regressing epithelium (Fig. 2A); (Jones et al., 1995). Involution is also accompanied by the expression of positive and negative regulators of the cell cycle including c-fos/c-jun (AP-1), c-myc, p53, and TGF-β (Strange et al., 1992; Marti et al., 1994; Marti et al., 1999). Therefore, the appearance of TN-C in the normal differentiated mammary gland during involution occurs in conjunction with loss of the basement membrane, suppression of milk protein synthesis, activation of immediate-early and cell cycle genes, and ultimately apoptosis. In contrast, increasing amounts of epithelial and stromal TN-C are generally observed in association with proliferating cells in mammary tumors that are progressing from the benign to the malignant state (Chiquet-Ehrismann, 1986; Mackie et al., 1987; Ferguson et al., 1990; Gould et al., 1990; Nicolo et al., 1990; Koukoulis et al., 1991; Moch et al., 1993; Natali et al., 1993).

Cultivating mammary epithelial cells on specialized three-dimensional extracellular matrices has provided many clues to explain the potential function of TN-C during adult mammary gland development. When primary cells or established cell lines and strains from mid-pregnant mice are maintained on a predominantly laminin-rich basement membrane in serum-free medium supplemented with lactogenic hormones, three-dimensional spherical alveolar-like structures form that resemble the alveolus in vivo (Barcellos-Hoff et al., 1989). This morphogenetic process is accompanied by down-regulation of TN-C, TGF-β1, c-myc, and cyclin D1, a lack of retinoblastoma hyperphosphorylation, and high-level expression of the cell cycle inhibitor p21 (Streuli et al., 1993; Jones et al., 1995; Boudreau et al., 1996). The quiescent cells forming each alveolus are polarized with basal nuclei and apical microvilli and are capable of synthesizing and secreting milk proteins, including β-casein. This ability to produce milk proteins first requires cellular rounding and clustering and thereafter β1 integrin clustering and increases in tyrosine phosphorylation (Roskelley et al., 1994). In the absence of this two-tier signaling hierarchy (i.e., after cultivation in two dimensions on tissue culture plastic), mammary cells synthesize high levels of TN-C and fail to produce milk (Fig. 2B) (Chammas et al.,1994; Jones et al., 1995).

Thus, like the mammary gland in vivo, TN-C is upregulated in the absence of a functional basement membrane, indicating that it may actually suppress milk protein synthesis. To test this, mammary cultures were treated with laminin-rich basement membrane together with intact or recombinant TN-C peptides encompassing different FN type III or fibrinogen homology domains. Intact TN-C (Fig. 2C), the FN type III domains 1–3, and the alternatively spliced A–D domains of TN-C inhibited β-casein milk protein synthesis, yet this had no short-term effects on cell viability and alveolar morphology. Additional studies showed that TN-C suppresses β-casein at the mRNA level and is able to interfere with activation of a 5′ ECM-responsive element that exists in the bovine β-casein gene (Schmidhauser et al., 1990; Schmidhauser, 1992; Jones et al., 1995). Collectively, these data suggest that TN-C may compete at the cell surface for receptors involved in the second tier of basement membrane-dependent signaling (i.e., integrin clustering and phosphorylation), rather than at the level of cell morphology.

It is well established that conflicting growth and differentiation signals initiate apoptosis. For instance, expression of the growth-promoting E1A adenoviral gene in serum-free conditions or in conjunction with expression of the p53 tumor suppressor gene induces apoptosis (Evan et al., 1992; Debbas and White, 1993). Similarly, forced overexpression of c-myc in basement membrane-dependent, three-dimensional differentiated alveolar structures, or overexpression of p21 in proliferating cells, initiates mammary epithelial cell apoptosis (Boudreau et al., 1996). Consistent with its appearance in the involuting mammary gland, chronic treatment of p21-expressing three-dimensional differentiated mammary epithelial cells with TN-C induces c-myc, interleukin-β1 converting enzyme (ICE) and apoptosis (Boudreau et al., 1996). On the other hand, transformed mammary epithelial cells are refractory to basement membrane-derived signals,

and by definition, are unable to form organized, three-dimensional structures and to differentiate (Petersen et al., 1992; Howlett et al., 1995). This principle is highlighted by a study in which treatment of mammary tumor cells in three-dimensional culture with inhibitory β1 integrin antibody or its Fab fragments led to morphological and functional reversion both in tissue culture and in vivo (Weaver et al., 1997). However, without the impediment of conflicting basement membrane-derived differentiation signals, it is likely that induction of cell cycle mediators, including c-myc by TN-C derived from the myoepithelium or stroma, would contribute to the ability of the adjacent epithelium to progress freely through the cell cycle. Indeed, transformed mammary cells maintained on two-dimensional TN-C substrates are able to proliferate, whereas suppression of TN-C with antiprogestins results in growth arrest and tumor differentiation (Chiquet-Ehrismann, 1986; Vollmer et al., 1992).

Mechanisms of Growth Control by Tenascin-C

In addition to the transformed mammary epithelium, numerous in vivo and tissue culture studies indicate that when expressed in the context of appropriate accessory factors, TN-C positively regulates cell proliferation using distinct cell surface receptors and signal transduction pathways (Table I). Activation of the TN-C gene promoter and the acquisition of the transformed phenotype after transient transfection of the c-jun proto-oncogene in ras-expressing fibroblasts also points to a direct role for this ECM component in modulating cell growth (Mettouchi et al., 1997). As a corollary to this, gene knockout studies using antisense/ribozyme constructs expressed in TN-C producing hypertrophied pulmonary arteries, or treatment of vascular smooth muscle cell cultures with TN-C antisense oligonucleotides suppresses growth and induces apoptosis. This suggests within remodeling adult vascular tissues, TN-C might act as both a growth and survival factor (Cleek et al., 1997; Cowan et al., 2000). An important observation that parallels this study is that radiolabeled anti-TN-C antibodies are able to induce regression of early and advanced grade human gliomas that express high levels of stromal TN-C (Bigner et al., 1998; Paganelli et al., 1999; Riva et al., 1999).

How does TN-C control cell growth? Classic studies by Folkman recognized that cell spreading on the ECM is required for proliferation in response to growth factors (Folkman and Moscona, 1978). Also, the use of sophisticated ECM-coated micropatterned surfaces have definitively shown that cell shape per se, rather than the extent of ECM-integrin binding, ultimately dictates whether a given cell will proliferate or undergo apoptosis (Chen et al., 1997). In light of this, it is not entirely surprising that a number of the growth-related signal transduction pathways initiated by TN rely on its ability to reorganize the actin-based cytoskeleton in a way that promotes cell spreading (Table I). For example, when cultured on type I collagen gels supplemented with TN-C, vascular smooth muscle cells spread extensively but have no growth advantage compared with their stellate-shaped counterparts maintained on collagen alone (Jones et al., 1997a). However, an examination of F-actin, EGF receptors, and tyrosine phosphorylated proteins demonstrates that TN-C promotes clustering of these proteins within focal adhesions in a manner that depends on the αvβ3 integrin receptor (Jones et al., 1997a). Addition of EGF to these TN-C "primed" cells leads to increased EGF receptor activation and the onset of growth (Jones and Rabinovitch, 1996; Jones et al., 1997b). Thus, cellular interactions with TN-C via the αvβ3 integrin receptor may support local cross-talk between integrins and receptor tyrosine kinases on actin-based scaffolds within focal adhesions. In terms of the specific domains and the downstream pathways involved in mediating this growth response, studies with SW480 carcinoma cells show that αvβ3 interactions with the third FN type III repeat supports cell spreading and proliferation. This finding depends on extracellular and cytoplasmic domains of β-integrin subunits and is associated with phosphorylation of focal adhesion kinase (FAK), paxillin, and ERK2 MAPK (Yokosaki et al., 1996). Because activation of ERK MAPKs and the onset of cell growth depends on both the ECM and soluble growth factors (Zhu and Assoian, 1995; Renshaw et al., 1999; Roovers et al., 1999), it is interesting that earlier studies with NIH-3T3 cells were unable to detect differences in the activity of receptor tyrosine kinases, despite the onset of growth (End, 1992). This finding suggests that TN-C may use alternative intracellular pathways and different TN receptors to activate growth in normal and transformed cells.

In contrast to studies relating TN-mediated cell spreading with the potentiation and promotion of growth, other work has shown that TN-C disrupts focal adhesions and the actin-based cytoskeleton, induces cellular rounding, and as such may be considered counteradhesive (Spring et al., 1989; Murphy-Ullrich et al., 1991; Prieto et al., 1992; Chung et al., 1996; Chung and Erickson, 1997; Fischer et al., 1997). This is not entirely inconsistent with a growth-modulatory role for TN-C, however, because cell growth and division presumably require differential and local detachment and reattachment of cells to the ECM. Nevertheless, a handful of studies have clearly shown that TN-C is able to inhibit cell proliferation, potentially via its effects on intracellular pH and cellular rounding (Crossin, 1991; Krushel et al., 1994). In this instance, TN-C may act to check runaway cell proliferation in actively remodeling tissues, thereby allowing tissues to reestablish homeostasis after a phase of growth. Additional prospective studies examining both the temporal and spatial patterns of TN expression and markers of cell proliferation in the context of other ECM proteins and soluble factors will be needed to clarify these issues. Also, a comparative analysis of the effect of TN on cell growth in tissue culture might help answer a number of unre-

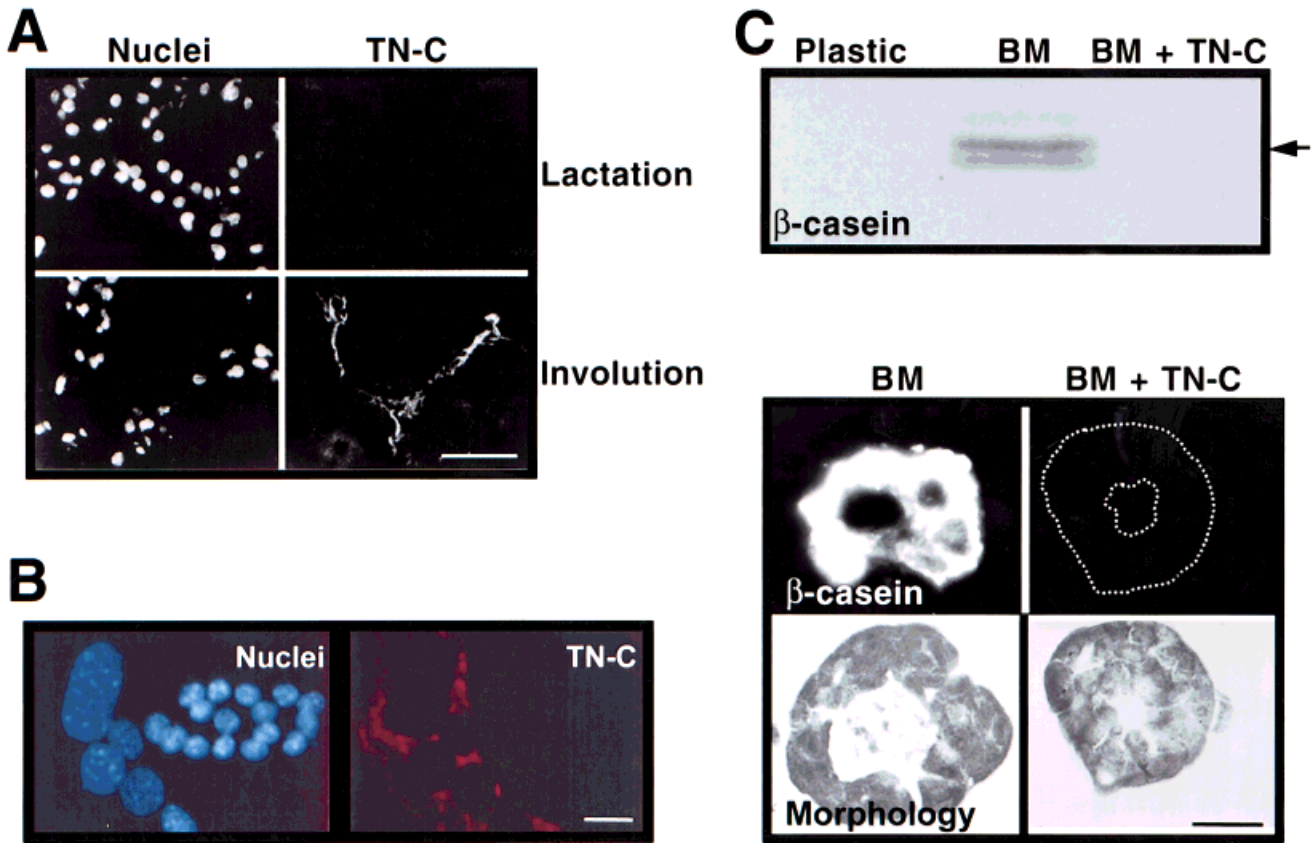

Fig. 2. Regulation and function of TN-C in the adult mammary gland. **A:** Immunofluorescence microscopy in mouse mammary gland indicates that TN-C protein is not synthesized in the functional, milk-producing mammary gland during lactation but is deposited around the epithelium at the onset of involution (right panel). This indicates that TN-C expression is incompatible with the milk-producing phenotype in vivo. Nuclei are stained with DAPI (left panel). **B:** The CID-9 mammary cell strain is unable to produce milk proteins on tissue culture plastic, even in the presence of lactogenic hormones. Under these conditions, the larger myoepithelial-like cells express TN-C protein, whereas the smaller epithelial cells are TN-C negative. Nuclei are stained with DAPI, which indicates that TN-C expression is incompatible with the milk-producing phenotype in tissue culture. **C:** Immunoprecipitation experiments show that when cultivated on tissue culture plastic in the presence of lactogenic hormones, mammary epithelial cells fail to express the milk protein β-casein (upper panel). In the presence of basement membrane, epithelial cells suppress TN-C expression (not shown), form alveolar-like structures, and express β-casein, as determined by immunoprecipitation (upper panel) and immunofluorescence microscopy. Cotreatment of cultures with basement membrane and TN-C protein (5 μg/ml) suppresses β-casein expression, yet this has no short-term effects on alveolar morphology. The structure of the alveolus on basement mebrane with TN-C is outlined by a broken white line. Adapted and reproduced from Jones et al. (1995) with permission of the Company of Biologists Ltd. Scale bar = 50 μm in A, 20 μm in B, 45 μm in C.

solved questions. (1) Is there a cell-type specific growth response to TN? (2) Does TN produce the same or different effects on growth when presented at different stages of the cell cycle, independent of cell type? (3) Does TN exert different functions depending on the constellation of other ECM proteins present (e.g., type I collagen)? These studies would also have to take into account the fact that various isoforms of TN produced by alternative splicing appear to correspond to different states of growth (Jones and Rabinovitch, 1996; Meiners and Geller, 1997) and that different TN-C receptors (αvβ3, α9β1 and αvβ6) interacting with same TN-C FN-III domain appear to produce dramatically different effects on cell morphology and growth (Yokosaki et al., 1996).

EXPRESSION OF TENASCINS

The rate of TN synthesis of the tenascins changes greatly in many cell types during embryogenesis. This property, coupled with their effects on cellular behavior, suggests that tenascin-containing matrices might orchestrate tissue morphogenesis by determining whether cells adhere to a substratum or to other cells in the same tissue, or by providing a provisional matrix that is conducive for cellular migration, division, or apoptosis. A key goal in studying TN biology is, therefore, to identify molecules and regulatory pathways that govern local expression of the tenascins.

TN-C expression is first observed at gastrulation and somite formation in a series of rostral-caudal waves that mirror temporal growth gradients that pass

through the chicken embryo (Crossin et al., 1986). At a later stage of somitogenesis, expression of TN-C was restricted to the rostral half of the somite during invasion of neural crest cells (Tan, 1987). Later during development, TN-C is produced by glia in the CNS. Its expression is particularly prominent on radial and Bergmann glial fibers during neuronal differentiation and migration in the cortex and cerebellum (Crossin et al., 1986; Yuasa, 1996). In the peripheral nervous system, TN-C is expressed by Schwann cells during myelination of peripheral nerves. Outside of the nervous system, TN-C is expressed during morphogenesis of the skeleton, connective tissue, and vasculature (Chiquet and Fambrough, 1984b). TN-C is produced at cellular borders where epithelial/mesenchymal transformations occur (Ekblom and Aufderheide, 1989), particularly in tissues undergoing branching morphogenesis (Tucker, 1991). In the adult, TN-C expression is induced at sites of neovascularization and wound healing (Mackie et al., 1988) and is induced by mechanical stress both in culture (Chiquet and Wehrle-Haller, 1994) and in vivo (Jrvinen et al., 1999). This is discussed more fully below. TN-C is also upregulated during pathological conditions including vascular hypertension and in the stroma surrounding tumor formation and metastases (Chiquet-Ehrismann, 1986; Mackie et al., 1987; Jones et al., 1997a).

Other members of the tenascin family are coexpressed with TN-C in several tissues, raising the possibility that combinations of TNs may account for several biological effects. For instance, both TN-C and TN-X are expressed in connective tissues, tendons, dermis, heart, kidney, vascular smooth muscle, and by astrocytes and Schwann cells during neural development (Burch et al., 1995; Geffrotin et al., 1995; Hasegawa et al., 1997). TN-Y is also coexpressed with TN-C in cells of the lung, skin, and in the kidney (Hagios et al., 1996; Hagios et al., 1999). Although expression of different TNs overlap in certain tissues, they frequently occur at different developmental schedules. For instance, although TN-C and TN-R are both expressed by CNS glia, TN-C appears earlier in development on radial glia during neuronal migration, whereas TN-R is expressed later during development by oligodendrocytes during the process of myelination.

In some developmental milieus, however, expression of tenascins is complementary, suggesting alternative control of gene transcription by growth factors for the particular tenascins exhibiting these expression patterns. For instance, during development of the skin and digestive tract, TN-X expression is complementary to that of TN-C (Matsumoto et al., 1994). Although TN-C and TN-Y are both expressed during CNS development, these molecules exhibit different cellular expression patterns: unlike TN-C that is expressed primarily by glia, TN-Y is expressed within the ependymal cells of the roof and floor plates (Tucker et al., 1999). TN-Y and TN-R also show little overlap in expression in the adult (Hagios et al., 1996; Hagios et al., 1999). These examples of contrasting cellular expression patterns for the different tenascins support the idea that distinct regulatory mechanisms control the expression of genes encoding these molecules.

The functions of the tenascins are better understood when TN expression patterns are related to expression patterns of some of the TN function-modulating ligands (Table 1). For instance, F11/contactin and TN-C are colocalized in the neural retina during synapse formation (D'Alessandri et al., 1995). TN-C, TN-R, and brevican are coexpressed during formation of perineuronal nets (Hagihara et al., 1999). Versican and TN-R are colocalized in the granular layer of the cerebellum during neuronal migration. In precartilaginous mesenchyme, versican and TN-C are coexpressed during its condensation, shaping, and differentiation (Perides et al., 1993). TN-R and the myelin-asssociated glycoprotein (MAG) are coexpressed in oligodendrocytes during myelination (Yang et al., 1999). Finally, both TN-C and syndecan are found at sites of epithelial-mesenchymal interaction during branching morphogenesis (Thesleff et al., 1987; Vainio et al., 1989). The coordinated expression of tenascins with several important ECM and cell surface ligands is likely to be governed by local growth and differentiation factors. Moreover, differences in expression patterns for the TNs and particular ligands indicate a combinatorial diversity in the regulatory elements that control particular aspects of TN expression.

Control of Tenascin Expression by Growth Factors

Transient expression of tenascin in both development and disease is induced by a variety of polypeptide growth factors. Early studies showed that synthesis of TN-C by embryonic fibroblasts could be upregulated by fetal bovine serum and TGFβ-1 (Pearson et al., 1988). Growth factors also dramatically upregulate TN-C expression in a number of neural tumor cell lines. The U251MG glioma cell line expresses high levels of TN-C constitutively (Erickson and Bourdon, 1989), whereas a number of undifferentiated neural tumor lines show differential regulation of TN-C in the presence of particular growth factors. For instance, fibroblast growth factor-2 (FGF-2) and the phorbol ester TPA stimulate TN-C expression in a Ewing's sarcoma-derived cell line, whereas only TPA stimulates TN-C production in the LAI55 neuroblastoma line. The induction of TN-C synthesis in both of these tumor cell lines was eliminated by actinomycin D and cycloheximide treatment, indicating that it required de novo RNA and protein synthesis (Rettig et al., 1994). These studies prompted investigation of the regulatory elements and transcription factors that induce TN-C transcription by growth factors, and by other factors that might regulate specific contexts of TN-C expression during development and disease.

Regulatory Elements That Control Expression of TN Genes

Understanding the regulatory mechanisms that control the different contexts of TN gene expression has been facilitated by the identification of TN-C promoters from several different species. These promoters are useful platforms to dissect signals arriving from the extracellular milieu, such as those that are associated with cell proliferation and mechanotransduction by integrins.

A substantial connection has already been forged between homeobox gene control and the expression of CAMs of the immunoglobulin superfamily (Edelman and Jones, 1998). Based on the segmented appearance of TN-C in the chicken embryo (Crossin et al., 1986) and identification of homeodomain binding sites within the chicken TN-C promoter (Jones et al., 1990), an attractive hypothesis was that local patterns of TN-C expression were controlled by different homeobox gene products. This would link genetic control of pattern formation by an important family of transcription factors to epigenetic information that is specified in part by ECM proteins.

To investigate whether homeobox gene products regulate activity of the TN-C promoter and to define promoter elements that responded to such cues, different TN-C promoter-reporter gene constructs were cotransfected into NIH-3T3 cells together with plasmids expressing particular homeobox genes (Jones et al., 1992). In the initial experiments, Evx-1 (a mouse homeobox protein most related to that encoded by the even-skipped pair-rule segmentation gene from *Drosophila*) stimulated chicken TN-C promoter activity via an 89-bp region of the TN-C promoter. This region of the promoter contained a TRE/AP-1 sequence, and mutation of this sequence eliminated Evx-1 activation of the TN-C promoter. TRE/AP-1 elements bind transcription factors of the Fos and Jun families and constitute targets of signal transduction pathways that are activated by growth factors (Angel et al., 1987; Curran and Franza, 1988). Therefore, activation of the TN-C promoter by Evx-1 is likely to mimic a growth factor response, involving a modulation of either the activity or expression of Fos/Jun proteins that interact with the TRE/AP-1 element. This regulatory strategy also appears to have been evolutionarily conserved: a TRE-like element within the proximal promoter of the human TN-C gene is required for upregulation of TN-C in response to cjun-mediated transformation of ras-activated rat embryonic fibroblasts (Mettouchi et al., 1997). Together, these observations strengthen the idea that TRE/AP-1 elements and Fos/Jun proteins are likely to play a key role in the activation of TN-C by growth factors.

Further support for evolutionarily conserved regulatory mechanisms controlling TN-C gene regulation is provided by the observations that the proximal promoter sequences of the chicken, mouse, and human TN-C genes have remarkable similarities (Jones et al., 1990; Copertino, 1995; Gherzi et al., 1995a; Gherzi et al., 1995b; Copertino et al., 1997). Several of the functional regulatory elements within TN-C promoters have been conserved, including binding sites for POU-homeodomain proteins, nuclear factor-1, NF-κB, AP-1 (Fos/Jun proteins), Krox/EGR-1 proteins, and an ATTA motif immediately upstream of the TATA box that binds to *Antennapedia*- and paired-related homeodomain proteins. In addition, glucocorticoids negatively regulate expression of mouse TN-C, and a binding site for glucocorticoid receptors has also been found in the chicken TN-C promoter sequence at −985 bp from the transcription start site (Ekblom et al., 1993). In addition to DNA elements that appear to have been conserved, some TN-C promoter elements appear to be species-specific. For instance, a binding site for two homeodomain proteins of the bicoid class, Otx1 and Otx2, are only found in the proximal region of the human TN-C promoter (Gherzi et al., 1997).

The promoter regions of TN-R and TN-X genes have also been identified, and experiments are underway to define elements that function in particular cellular contexts (Carnemolla et al., 1996; Leprini et al., 1996; Speek et al., 1996; Gherzi et al., 1998). However, it is currently not known whether certain regulatory mechanisms have been conserved in all TN genes or whether others have arisen to function in regulatory contexts that are unique to a particular tenascin. Nevertheless, it is likely that such experiments and comparisons will reveal an evolutionarily conserved mechanism for the control of TN family genes by homeobox transcription factors in development and disease. Indeed, as is described in more detail below, binding sites for homeobox proteins in the TN-C promoter play a vital role in the induction of TN-C expression in pulmonary vascular remodeling and disease.

Reciprocal Regulation of MMPs and TN-C

Within certain developing and remodeling adult tissues, including heart valves, mammary gland, elastic arteries and angiogenic blood vessels, TN-C deposition coincides with the expression and activity of matrix metalloproteinases (MMPs) (Hedin et al., 1991; Talhouk et al., 1991; Talhouk et al., 1992; Bendeck et al., 1994; Zempo et al., 1994; Canfield and Schor, 1995; Jones et al., 1995; Jones et al., 1999b; Koivunen et al., 1999; Cowan et al., 2000). This raises the possibility that TN-C induces MMP expression and activity, which in turn would edit the preexisting ECM to provide a modified tissue microenvironment that is conducive for cell motility, growth, survival, and/or differentiation. Alternatively, MMP-mediated catabolism of the preexisting ECM might promote the formation of a TN-C-enriched provisional matrix that supports cell adhesion and alternative signaling pathways and functions. For specific mechanistic details regarding the signaling functions of MMPs, the reader is referred to a number of excellent reviews (Birkedal-Hansen, 1995; Basbaum

and Werb, 1996; Streuli, 1999). In support of a role for TN-C in regulating MMPs, when rabbit fibroblasts derived from the synovium are plated on a mixed substrate of fibronectin and TN-C, expression of collagenase, stromelysin, the 92-kDa gelatinase (MMP-9), and nuclear c-fos is upregulated (Tremble et al., 1994). Similarly, MMP-2 mRNA expression is induced in heart valve interstitial cells cultivated on mixed substrates of type I collagen and TN-C, but not collagen alone (Li et al., 1999). For synovial fibroblasts, the combined effect of fibronectin and TN-C on MMP expression can be inhibited by the TN-68 monoclonal antibody that recognizes an epitope within the seventh and eighth FN-III repeat of TN-C, but not by TN M1, a monoclonal antibody that recognizes an epitope in the EGF domains (Tremble et al., 1994). Furthermore, addition of soluble TN-C to cells plated on fibronectin had no effect on MMP expression, thereby indicating that solid-state interactions with fibronectin are required. This result is consistent with the finding that soluble versus substrate-bound TN-C have contrary effects on cell behavior (Table 1) and highlights the need to reevaluate the role of TN-C in alternative contexts provided by specialized tissue-specific extracellular matrices.

Another requirement for induction of MMPs by synovial fibroblasts and heart valve interstitial cells is the acquisition of an appropriate cell shape (Aggeler et al., 1984; Tremble et al., 1994; Li et al., 1999). For synovial fibroblasts, cellular rounding and an intact actin cytoskeleton are prerequisites for induction of MMPs by TN-C and fibronectin (Aggeler et al., 1984; Tremble et al., 1994). Increased MMP-2 expression in heart valve interstitial cells, however, is accompanied by increased cell spreading (Li et al., 1999). A recent investigation of genes induced by TN-C in heart valve interstitial cells identified the actin-modifying protein thymosin β4 as one downstream target and further showed that treatment of interstitial cells with thymosin β4 peptides mimics the effect of TN-C on cell spreading and MMP-2 production (Li et al., 1999). It would, therefore, be of interest to determine whether the ability of TN-C to modify F-actin, focal adhesions, and associated structures, including growth factor receptors and integrins, is also mediated via thymosin β4. This may be relevant to a number of other biological processes, including angiogenesis and epidermal wound healing, in which thymosin β4 and TN-C have been shown to play a supportive role (Whitby and Ferguson, 1991; Canfield and Schor, 1995; Latijnhouwers et al., 1996; Zagzag et al., 1996; Malinda et al., 1997; Malinda et al., 1999; Schenk et al., 1999).

Alterations in cell adhesion and shape dictated by the ECM not only influence catabolism of the supporting ECM, but also expression of the ECM itself (Hedin et al., 1988; Streuli et al., 1991; Chiquet-Ehrismann et al., 1994; Jones et al., 1995; Jones et al., 1997a). For example, disruption of the F-actin cytoskeleton with cytochalasin D inhibits basal levels of TN-C gene promoter activity in chick embryo fibroblasts cultivated on type I collagen (Chiquet-Ehrismann et al., 1994). Cell spreading by the CID-9 mammary epithelial cell strain on tissue culture plastic in the presence of lactogenic hormones is characterized by increased steady-state levels of TN-C mRNA and the absence of tissue-specific markers including the milk protein β-casein (Jones et al., 1995). In contrast, basement membrane-induced cellular rounding in CID-9 was accompanied by suppression of TN-C mRNA and induction of β-casein synthesis (Jones et al., 1995). These data may explain in part how TN-C is upregulated during adult mammary gland involution in vivo, a process characterized by MMP-mediated destruction of the basement membrane and the loss of milk protein synthesis (Talhouk et al., 1991; Sympson et al., 1994). Direct evidence indicating that MMP-mediated remodeling of the basement membrane regulates TN-C is derived from transgenic mouse studies in which inappropriate expression of an autoactivated form of stromelysin-1 in the mammary epithelium leads to the formation of a reactive stroma that is enriched with TN-C (Thomasset et al., 1998).

Connecting MMPs and Integrin Signaling to Elements in the TN-C Promoter

Akin to the mammary epithelium, vascular smooth muscle cell TN-C gene expression is also regulated by MMPs (MMP-2) (Jones et al., 1997a; Jones et al., 1997b; Cowan et al., 1999a; Cowan et al., 2000). For example, when vascular smooth muscle cells are cultivated on native type I collagen, a β1 integrin ligand, TN-C gene expression is suppressed (Jones et al., 1997a; Jones et al., 1999a), whereas on denatured or MMP-proteolyzed type I collagen, a β3 integrin ligand (Davis, 1992; Tuckwell et al., 1994; Messent et al., 1998), TN-C gene expression is induced (Jones et al., 1999a). Moreover, increased TN-C synthesis on denatured collagen is associated with elevated protein tyrosine phosphorylation, including activation of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2), whereas β3 integrin function-blocking antibodies inhibit this effect (Fig. 3A). Consistent with these findings, treatment with the specific MEK inhibitor, PD 98059, results in suppression of TN-C protein synthesis (Fig. 3A). To investigate whether β3 integrin-dependent activation of ERK1/2 regulates the tenascin-C promoter, an approximately 4-kb mouse tenascin-C gene promoter-chloramphenicol acetyltransferse reporter construct was introduced into cells on native and denatured collagen. This showed that relative to native collagen, TN-C promoter activity is increased on denatured collagen (Fig. 3B).

Next, to identify regions of the promoter involved, a series of TN-C promoter constructs with 5′ deletions and showed that denatured collagen-dependent promoter activity was retained by a 122-bp element, located −43 to −165 bp upstream of the RNA start site. Activation of this element was suppressed either by blocking β3 integrins or by preventing ERK1/2 activation (Fig. 3C and D). These observations demonstrate

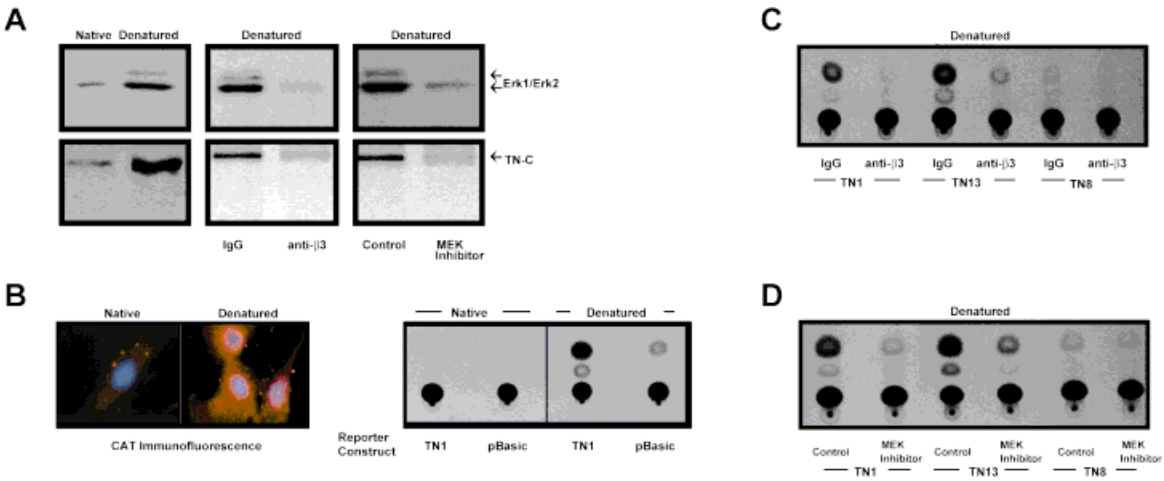

Figure 3.

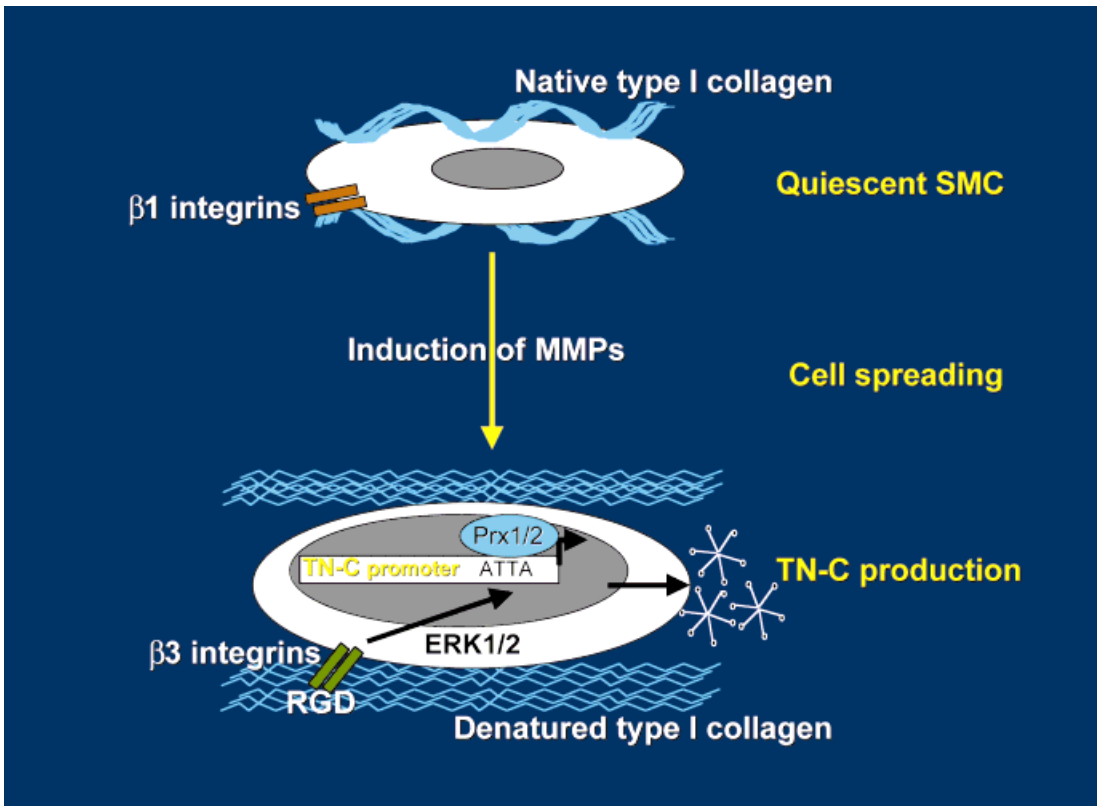

Fig. 4. Hypothetical scheme showing how denatured type I collagen might drive vascular smooth muscle cell TN-C gene. On native type I collagen, vascular smooth muscle cells attach and spread by using β1 integrins. Under serum-free conditions, these cells are quiescent. Degradation of type I collagen by matrix metalloproteinases leads to increased cell spreading, exposure of previously cryptic RGD sites in type I collagen, and upregulation of prx1 and prx2. The β3 integrins activate ERK1 and ERK2 MAPKs, which modify prx1 and prx2. In turn, activated prx1 and/or prx2 interact with an ATTA "ECM-responsive element" in the TN-C gene promoter, located at position −57 bp upstream from the transcription start site. This interaction induces TN-C transcription leading to increased deposition of TN-C protein in the vascular ECM.

that smooth muscle cell adhesion to denatured collagen via β3 integrins activates the mitogen-activated protein kinase pathway, which is required for the induction of tenascin-C gene expression using a 122-bp "extracellular matrix response element" in the TN-C gene promoter. (Jones et al., 1997a; Jones et al., 1999a). Site-directed mutagenesis studies have further defined an ATTA homeodomain-binding site located at position −57 bp as being critical for this ECM response. Prx1 (Mhox) and prx2 (S8) encode paired-related homeodomain-containing transcription factors that interact with A/T-rich gene sequences in vitro (Cserjesi et al., 1992; de Jong et al., 1993; Cserjesi et al., 1994; Brickell, 1995; Hu et al., 1995; Leussink et al., 1995; Bergwerff et al., 1998; Lu et al., 1999), yet little is known either about their regulation or target genes, especially in vivo. Denatured collagen induces smooth muscle cell prx1 and prx2 mRNA expression, whereas overexpression of each of these factors activates the TN-C gene promoter in prx1/2-null smooth muscle cells (Gates et al., 1999). The potential interactions between prx1 and prx2 and the TN-C promoter are likely to be relevant to vascular disease because they are upregulated and colocalize with TN-C during the onset and progression of pulmonary vascular disease in proliferating vascular smooth muscle cells and adventitial fibroblasts in the adult rat (Jones and Rabinovitch, 1996; Gates et al., 1999). Paired-related homeodomain proteins that are normally used in genetic programming contexts during development may, therefore, respond to MMP-mediated changes in the adult vascular ECM by increasing TN-C production (Fig. 4). In light of these new findings, it would be of interest to determine whether MMPs, prx1 and prx2 might also regulate TN-C expression in heart valves, developing bone and scarless wounds, within which each of these factors has already been implicated to play a functional role.

Biomechanical Regulation of TN-C

Most cells are constantly exposed to mechanical forces that modify and maintain their biological behavior (Gooch and Tennant, 1997). A number of studies indicate that mechanical signals are not only detected by the ECM and associated cell adhesion receptors including integrins, but that intracellular signals generated by these receptors may modulate cell adhesion components used to survey and interact with this mechanical microenvironment. For example, stretching human umbilical endothelial cells (HUVECs) increases β3 integrin mRNA levels and leads to reorganization of β1, α5, and α2 integrins in a linear pattern (Suzuki et al., 1997; Yano et al., 1997). Subclavian pulmonary artery anastomoses in rat resulting in systemic-like hemodynamics in the pulmonary vasculature enhances monocrotaline-induced pulmonary vascular disease, characterized by increased expression of tropoelastin, type I procollagen, and TN-C (Jones and Rabinovitch, 1996; Tanaka et al.,1996). Similarly, the hemodynamic stress of increased arterial pressure induces TN-C expression in saphenous vein grafts interposed into the arterial circulation (Wallner et al., 1999).

Additional evidence demonstrates that TN-C gene expression is mechanosensitive. Mechanical strain induces TN-C in neonatal rat cardiac myocytes in an amplitude-dependent manner (Yamamoto, 1999), whereas chick embryo fibroblasts, vascular smooth muscle cells, and whole pulmonary arteries cultured on attached type I collagen substrates produce higher levels of TN-C than those cultivated on mechanically relaxed collagen gels (Chiquet-Ehrismann et al., 1994; Jones et al., 1997a; Cowan et al.,1999). Physical loading and the resulting increased strain imposed on the rat ulna leads to early increases in osteoblast TN-C

Fig. 3. Identification of an ECM-responsive element in the TN-C promoter. **A:** Immunoblot analysis of A10 vascular smooth muscle cell lysates demonstrates that denatured type I collagen supports sustained activation (phosphorylation) of ERK1 and ERK2, whereas native collagen suppresses this activity (A, left panel). Total levels of ERK1 and ERK2 are identical under these different conditions (not shown). Treatment of A10 cells on denatured collagen with an anti-β3 integrin function-blocking antibody (A, center panel) or with an ERK1 and ERK2 inhibitor (PD 98059, which inhibits MEK) (A, right panel) results in a loss of ERK1/2 activity. Parallel immunoprecipitation experiments show that TN-C protein synthesis increases on denatured collagen (A, left panel) yet is suppressed after β3 integrin blockade (A, center panel) or after indirect inhibition of ERK1 and ERK2 activity with the MEK inhibitor (A, right panel). These data indicate that on denatured collagen, β3 integrin-dependent activation of ERK1 and ERK2 regulates TN-C in vascular SMCs. **B:** The activity of a CAT reporter gene driven by a 4,028-bp mouse TN-C gene promoter (TN1-CAT) was examined in A10 cells after transient transfection on native or denatured type I collagen. Controls were transfected with pCATbasic (the promoterless parent vector). Immunofluorescent staining for CAT protein in TN1-CAT transfected cells on native collagen reveals low levels of CAT protein, whereas on denatured collagen, high levels of CAT expression are observed (B, left panel). Direct assessments of TN1-CAT activity on native versus denatured collagen demonstrate that activation of the mouse TN-C promoter in cells cultured on native collagen produces CAT activity that is comparable with the pCAT parent plasmid (B, right panel). In contrast, denatured collagen significantly increases TN1-CAT activity (B, right panel). A 122-bp region of the TN-C located between −165 and −43 bp upstream from the transcription start site (contained in plasmids TN1-CAT and TN13-CAT but not in minimal TN-C promoter construct TN-8) responded to denatured type I collagen. **C,D:** To determine whether activation of the 122-bp denatured collagen-responsive element was dependent on β3 integrins and the activity of ERK1 and ERK2, A10 cells maintained on denatured collagen were transfected with TN-CAT constructs. CAT activity was then assessed after β3 integrin blockade with a function-blocking antibody or after inhibition of MEK with PD 98059. Compared with cultures exposed to control IgG, treatment with anti-β3 integrin antibodies reduces CAT activity derived from TN1-CAT and TN13-CAT to the same levels as TN8-CAT transfected cells (panel C). Inhibition of ERK1/2 activation with PD 98059 has a similar effect (panel D). These effects were achieved without compromising cell viability (not shown). Thus, denatured collagen activates the TN-C gene promoter in vascular smooth muscle cells via a β3 integrin and MAP kinase signaling pathway that involves activation of ERK1 and 2 and an ECM-responsive element in the TN-C gene promoter element. Adapted and reproduced from Jones et al. (1999a) with permission of the Company of Biologists Ltd.

expression (Webb et al., 1997). These findings are noteworthy because TN-C is expressed in the developing skeleton, and it regulates osteoblast differentiation in culture (Mackie and Tucker, 1992; Mackie and Ramsey, 1996). More recently, in vivo studies examining TN-C expression in fibroblasts and chondrocytes within the osteotendinous junction showed that removal of mechanical stress from the junction suppresses TN-C expression (Jrvinen et al., 1999). Applied pressure as a mechanical force also modulates TN-C expression and distribution during postburn scar remodeling; in this way it might accelerate the reparative process (Costa et al., 1999). Collectively, these studies indicate that TN-C expression is modulated by altered biomechanics and that this may potentially account for its appearance in other tissues where it is believed to play a regenerative or morphoregulatory role such as healing wounds and the tumor stroma (Mackie et al., 1988; Sakakura, 1991).

Most studies indicate that the control of TN-C expression by mechanical factors occurs at the level of the gene promoter and that different promoter motifs and transcription factors may be used, depending on cell type and the culture conditions used. For example, mechanical upregulation of TN-C in chick embryo fibroblasts cultivated in stressed collagen gels relies on a novel and conserved GAGACC stress response element (Chiquet et al., 1996; Chiquet, 1999).

In cardiomyocytes, induction of TN-C by mechanical strain depends on NF-kB and reactive oxygen species (Yamamoto, 1999). Interestingly, NF-kB and reactive oxygen species also regulate the production of MMPs by fibroblasts (Kheradmand et al., 1998), indicating that biomechanical induction of TN-C might occur secondary to MMP-mediated structural alterations in the preexisting ECM. In keeping with this idea, stress-unloading collagen gels containing vascular smooth muscle cells or intact pulmonary arteries suppresses MMPs and TN-C (Jones et al., 1997a; Cowan et al., 1999). Also, the TN-C promoter contains a consensus binding site for Krox-24/Egr-1, a zinc-finger transcription factor that was induced by mechanical factors including shear stress and cyclic strain (Khachigian et al., 1997; Schwachtgen et al., 1998; Houston et al., 1999; Morawietz et al., 1999; Wung et al., 1999). Chronic increases in pulmonary blood flow in neonatal pigs not only increase MMP-2 and MMP-9 expression and activity but also activates Krox-24/Egr-1 and TN-C mRNA and protein expression (Jones et al., 1999c). Likewise, cultivating SMC on denatured type I collagen in static tissue cultures recapitulates this flow-dependent increase in Krox-24/Egr-1-binding activity and TN-C expression (Jones et al., 1999c), once again indicating that biomechanical forces may regulate smooth muscle TN-C via their effects on catabolism of the vascular ECM.

TENASCIN KNOCKOUT MICE

In 1992, Saga et al. generated a TN-C knockout mouse by homologous recombination. These mice appeared normal and showed no tenascin expression. This observation was confirmed (Settles et al., 1997), after having been challenged (Mitrovic and Schachner, 1995). TN-C knockout mice were also generated independently by a different group of investigators (Forsberg et al., 1996), yielding conclusions that were similar to those from the original study (Saga et al., 1992), namely, that a lack of TN-C does not produce any gross anatomical abnormalities, and that TN-C is not essential for development. These initial observations were interpreted in a number of interesting ways. For instance, Erickson proposed that the complex developmental pattern of TN-C expression might be an example of superfluous gene expression that provides a detailed record of the evolutionary history of the regulation of this gene in vertebrates (Erickson, 1993).

Those working in the tenascin field had hoped that deletion of the TN-C gene would produce more than just a subtle phenotype. However, a molecular biologist's approach to development frequently engenders an oversimplified view of the role of genes. It is well known from genetic studies in *Drosophila* that there is not a one-to-one correspondence between genotype and phenotype. Moreover, it is inadequate to examine the effects of gene deletions without considering other genetic terms such as pleiotropy and variable penetrance, i.e., that the tenascins function in a network of other gene products that may or may not compensate for loss of TN-C, and although progress is being made in this arena, it is still poorly understood what constitutes a functional gene network vis-à-vis a particular developmental process. Explanations for the apparent dispensability of TN-C during development need not invoke compensatory expression by other molecules, although evidence for this now exists (Cowan et al., 2000). It is, nonetheless, unclear whether the ECM produced in TN-C knockout mice is truly normal in fitness of the animal and its resilience and elasticity when it interacts with its environment. The other problem is variable penetrance, and it appears that any gene knockout with subtle phenotypic effects must be analyzed in several genetic backgrounds. These and other caveats that apply to conclusions drawn from knockout experiments have been well stated by Crossin (1994).

The simple point remains that the TN-C knockout mice still have not been analyzed in enough detail to demonstrate developmental defects. This has been made clear in a recent review on the subject (Mackie and Tucker, 1999). In fact, the closer one looks, the more evident it becomes that TN-C knockout mice are far from normal. TN-C knockout mice have several neurological defects that result in abnormal behavior including hyperactivity in an open field test, poor sensorimotor coordination, clinging, and freezing behavior during bridge-crossing tasks, and poor performance in passive avoidance learning tests (Fukamauchi et al., 1996; Fukamauchi and Kusakabe, 1997; Fukamauchi et al., 1997; Fukamauchi et al., 1998). These behavioral deficits were only observed on a C57BL/6J-CBA back-

ground and not in the original 129SJ strain. A number of alterations in gene expression in neurons accompanied these changes in behavior. These included alterations in the levels of particular neurotransmitters and their biosynthetic enzymes, specifically down-regulation of tyrosine hydroxylase and neuropeptide Y synthesis and upregulation of preprotachykinin A and CCK (Fukamauchi and Kusakabe, 1997; Fukamauchi et al., 1997; Fukamauchi et al., 1998). For myelination, conclusions differ. One study (Moscoso et al., 1998) found that the size of nerve terminals at neuromuscular junctions and the capacity for reinnervation after nerve crush was relatively normal in the TN-C knockout compared with wild-type littermates, whereas another study (Cifuentes-Diaz et al., 1998) concluded that the TN-C knockout had a significantly reduced percentage of botulinum toxin-induced sprouting of processes in nerve terminals compared with control animals. A recent study (Kiernan et al., 1999) from another group confirmed the finding of abnormal behavior in TN-C null mice, including hyperlocomotion and deficits in coordination, but found no abnormalities in myelination patterns or in oligodendrocyte precursor migration. Collectively, these findings clearly indicate that mice lacking TN-C are deficient in behavioral processes. For regenerative processes, TN-C knockout mice show reduced corneal wound healing and hematopoiesis (Ohta et al., 1998; Talts et al., 1999) and are grossly deficient in regeneration from snake venom-induced glomerulonephritis (Nakao et al., 1998).

The first report on the TN-R knockout mouse indicates that the conduction along optic nerves is abnormal. Moreover, phosphacan (an important ligand of TN-R) is misexpressed in TN-R knockout mice and no longer appears in perineuronal nets (Weber et al., 1999). These alterations in neuronal conductivity and changes in ECM around synapses is likely to affect other physiological functions in the brain and might also lead to behavioral deficits that are similar to those observed in TN-C knockout mice. A cross of TN-C and TN-R knockout mice might reveal that a lack of both of these tenascins leads to a gross misrouting of neuronal connections and additional abnormalities in neuronal activity and plasticity.

PERSPECTIVES

Although a wealth of information exists about the structure, function, and regulation of TN-C, the recent discoveries of new TN family members will no doubt fuel new avenues of investigation. For instance, overlapping patterns of expression observed between different TN proteins in vivo might actually reflect the formation of TN chimeras. If TNs have the capacity to form heteromultimers, then coexpression of different family members adds an additional layer of complexity to the functions of TN-containing matrices. Although the use of individual TN domains has provided many clues about interactions and functions, this approach may not reveal the true nature of native TN molecules incorporated into a three-dimensional, tissue-specific ECM. It is also unclear whether high affinities of binding between TN and its ligands in vitro actually occur in vivo. In reality, the question about what constitutes a true ECM in vivo still remains unanswered. Tremendous strides have been made, however, to recreate three-dimensional tissue microenvironments comprised of multiple ECM proteins including TN proteins. This approach has certainly proved to be worthwhile in mammary epithelial and vascular biology and is important for understanding the role of TN in the assembly of functional macromolecular ECM networks. For example, colocalization of TN-R with members of the aggrecan family (particularly in perineuronal nets) results in the formation of a unique ECM architecture that may be biologically significant for neuronal conductivity.

The signal transduction pathways and responses initiated by TN binding to cell surfaces are highly complex and diverse in nature, which probably reflects the existence of numerous receptors that produce different signal inputs. In turn, these different signals result in differential gene expression, and as such, represent epigenetic information that has been converted into a genetic response. However, it must be mentioned that cellular interactions with TN or other ECM proteins via the same or distinct receptors may on occasion initiate identical signal transduction pathways (e.g., FAK and MAPKs). This leaves open the question as to how TN may produce different functions using identical pathways. This answer may lie with another function of the ECM, i.e., its unique ability to shape cells within tissues in a way that modifies the amplitude and duration of intracellular signaling through ubiquitous mediators such as FAK and ERK. This is especially striking in PC12 neuronal cells where transient bursts of ERK activity lead to cell proliferation, and sustained ERK activity results in differentiation. Similarly, low levels of Raf are accompanied by induction of cyclin D1 and proliferation, whereas higher sustained levels of Raf lead to induction of p21 and cell-cycle arrest. Coupled with recent findings demonstrating that the duration of ERK MAPK signaling is cell shape-dependent, it would be of interest to determine whether TN alters the processing of ubiquitous signals via its known effects on actin and cell shape.

Studying the dynamics of the TN-C promoter has led to the identification of several distinct functional elements. It is likely that combinations of these elements, together with cell-specific *trans*-factors lead to specific contexts of TN expression. For instance, although regulation of TN by homeobox proteins appears to be a general theme, responses at ATTA elements within the TN promoter will depend on the particular profile of homeobox proteins in a given cell type (its developmental history) in the background of the current cellular signaling state and shape. Here, we have illustrated such a pathway in vascular smooth muscle cells that starts with ECM integrin-dependent activation of ERK

MAPK, culminating in the activation of the TN-C promoter via an ATTA homeodomain-binding site in the TN-C promoter. These studies raise the possibility that certain homeobox genes that are normally associated with embryonic development may be re-activated in the adult organism, particularly in contexts of place-dependent tissue remodeling.

A rapidly emerging theme in tenascin biology is its regulation by biomechanical factors. It would be of interest to explore whether physical manipulation of TN-containing substrates (which exhibit highly elastic properties) also alters cellular signal transduction, perhaps by permitting particular ligand interactions that occur only when TN is stretched. Also, a reexamination of the patterns of TN expression during development and disease vis-à-vis local differentials in the force within a particular tissue would provide strong supportive in vivo evidence that TN is uniquely positioned to drive morphogenesis and help tissues adapt to physical stress.

Concerning the overall roles of TNs in vivo, it will obviously be important to analyze the null mutations of TNs other than TN-C and their effects on development and to cross these knockout mice to determine synergistic effects that occur in distinct developmental situations that lack TN. For instance, recent observations showing that TN-X gene deletions and recombinations lead to human congenital disorders and disease make this gene a particularly promising candidate for knockout in the mouse and may yield a recognizable phenotype with severe abnormalities in developmental patterning. However, care must be exercised not to overinterpret results because genes do not act alone, and until we understand how they function in meaningful morphological networks, it is premature to label a molecule as superfluous or dispensable. Irrespective of the outcome of such genetic experiments, an ample body of evidence already suggests that the elastic properties of tenascin glycoproteins in the arenas of connective tissue and neural plasticity benefit an organism in the wild as it adapts to its environment.

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REFERENCES

Aggeler J, Frisch SM, Werb Z. 1984. Changes in cell shape correlate with collagenase gene expression in rabbit synovial fibroblasts. J Cell Biol 98:1662–1671.

Akke M, Liu J, Cavanagh J, Erickson HP, Palmer AG III. 1998. Pervasive conformational fluctuations on microsecond time scales in a fibronectin type III domain. Nat Struct Biol 5:55–59.

Angel P, Imagawa M, Chiu R, Stein B, Imbra RJ, Rahmsdorf HJ, Jonat C, Herrlich P, Karin M. 1987. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated trans-acting factor. Cell 49:729–739.

Aspberg A, Adam S, Kostka G, Timpl R, Heinegard D. 1999. Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. J Biol Chem 274:20444–20449.

Aspberg A, Miura R, Bourdoulous S, Shimonaka M, Heinegard D, Schachner M, Ruoslahti E, Yamaguchi Y. 1997. The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. Proc Natl Acad Sci USA 94: 10116–10121.

Aukhil I, Joshi P, Yan Y, Erickson HP. 1993. Cell- and heparin-binding domains of the hexabrachion arm identified by tenascin expression proteins. J Biol Chem 268:2542–2553.

Baker NE, Mlodzik M, Rubin GM. 1990. Spacing differentiation in the developing Drosophila eye: a fibrinogen-related lateral inhibitor encoded by *scabrous*. Science 250:1370–1377.

Barcellos-Hoff MH, Aggeler J, Ram TG, Bissell MJ. 1989. Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. Development 105: 223–235.

Barnea G, Grumet M, Milev P, Silvennoinen O, Levy JB, Sap J, Schlessinger J. 1994. Receptor tyrosine phosphatase beta is expressed in the form of proteoglycan and binds to the extracellular matrix protein tenascin. J Biol Chem 269:14349–14352.

Baron M, Main AL, Driscoll PC, Mardon HJ, Boyd J, Campbell IA. 1992. ^{3}H NMR assignment and secondary structure of the cell adhesion type III module of fibronectin. Biochemistry 31:2068–2073.

Basbaum CB, Werb Z. 1996. Focalized proteolysis: spatial and temporal regulation of extracellular matrix degradation at the cell surface. Curr Opin Cell Biol 8:731–738.

Baumgartner S, Chiquet-Ehrismann R. 1993. Tena, a Drosophila gene related to tenascin, shows selective transcript localization. Mech Dev 40:165–176.

Baumgartner S, Martin D, Hagios C, Chiquet-Ehrismann R. 1994. Tenm, a Drosophila gene related to tenascin, is a new pair-rule gene. EMBO J 13:3728–3740.

Bell SC, Pringle JH, Taylor DJ, Malak TM. 1999. Alternatively spliced tenascin-C mRNA isoforms in human fetal membranes. Mol Hum Reprod 5:1066–1076.

Bendeck MP, Zempo N, Clowes AW, Galardy RE, Reidy MA. 1994. Smooth muscle cell migration and matrix metalloproteinase expression after arterial injury in the rat. Circ Res 75:539–545.

Bergwerff M, Gittenberger-de Groot AC, DeRuiter MC, van Iperen L, Meijlink F, Poelmann RE. 1998. Patterns of paired-related homeobox genes PRX1 and PRX2 suggest involvement in matrix modulation in the developing chick vascular system. Dev Dyn 213:59–70.

Bigner DD, Brown MT, Friedman AH, Coleman RE, Akabani G, Friedman HS, Thorstad WL, McLendon RE, Bigner SH, Zhao XG, Pegram CN, Wikstrand CJ, Herndon JE II, Vick NA, Paleologos N, Cokgor I, Provenzale JM, Zalutsky MR. 1998. Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: phase I trial results. J Clin Oncol 16:2202–2212.

Birkedal-Hansen H. 1995. Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol 7:728–735.

Bishop SA, Klein T, Arias AM, Couso JP. 1999. Composite signalling from Serrate and Delta establishes leg segments in Drosophila through Notch. Development 126:2993–3003.

Borsi L, Balza E, Castellani P, Carnemolla B, Ponassi M, Querz G, Zardi L. 1994. Cell-cycle dependent alternative splicing of the tenascin primary transcript. Cell Adhesion Comm 1:307–317.

Borsi L, Balza E, Gaggero B, Allemanni G, Zardi L. 1995. The alternative splicing pattern of the tenascin-C pre-mRNA is controlled by the extracellular pH. J Biol Chem 270:6243–6245.

Boudreau N, Werb Z, Bissell MJ. 1996. Suppression of apoptosis by basement membrane requires three- dimensional tissue organization and withdrawal from the cell cycle. Proc Natl Acad Sci USA 93:3509–3513.

Bourdon MA, Wikstrand CJ, Furthmayer H, Matthews TJ, Bigner DD. 1983. Human glioma-mesenchymal extracellular matrix antigen defined by monoclonal antibody. Cancer Res 43:2796–2805.

Brickell PM. 1995. MHox and vertebrate skeletogenesis: the long and the short of it. Bioessays 17:750–753.

Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL. 1993. Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. J Cell Biol 122:265–278.

Brummendorf T, Wolff JM, Frank R, Rathjen FG. 1989. Neural cell recognition molecule F11: homology with fibronectin type III and immunoglobulin type C domains. Neuron 2:1351–1361.

Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. 1995. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn 203:491–504.

Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. 1997. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 17:104–108.

Canfield AE, Schor AM. 1995. Evidence that tenascin and thrombospondin-1 modulate sprouting of endothelial cells. J Cell Sci 108:797–809.

Carnemolla B, Leprini A, Borsi L, Querze G, Urbini S, Zardi L. 1996. Human tenascin-R: complete primary structure, pre-mRNA alternative splicing and gene localization on chromosome 1q23-q24. J Biol Chem 271:8157–8160.

Carr PA, Erickson HP, Palmer AG III. 1997. Backbone dynamics of homologous fibronectin type III cell adhesion domains from fibronectin and tenascin. Structure 5:949–959.

Chammas R, Taverna D, Cella N, Santos C, Hynes NE. 1994. Laminin and tenascin assembly and expression regulate HC11 mouse mammary cell differentiation. J Cell Sci 107:1031–1040.

Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. 1997. Geometric control of cell life and death. Science 276:1425–1428.

Chiquet M, Fambrough DM. 1984a. Chick myotendinous antigen. I. A monoclonal antibody as a marker for tendon and muscle morphogenesis. J Cell Biol 98:1926–1936.

Chiquet M, Fambrough DM. 1984b. Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. J Cell Biol 98:1937–1946.

Chiquet M, Matthisson M, Koch M, Tannheimer M, Chiquet-Ehrismann R. 1996. Regulation of extracellular matrix synthesis by mechanical stress. Biochem Cell Biol 74:737–744.

Chiquet M, Wehrle-Haller B. 1994. Tenascin-C in peripheral nerve morphogenesis. Perspect Dev Neurobiol 2:67–74.

Chiquet M. 1999. Regulation of extracellular matrix gene expression by mechanical stress. Matrix Biol 18:417–426.

Chiquet-Ehrismann R, Kalla P, Pearson CA, Beck K, Chiquet M. 1988. Tenascin interfaces with fibronectin action. Cell 53:383–390.

Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. 1986. Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47:131–139.

Chiquet-Ehrismann R, Matsuoka Y, Hofer U, Spring J, Bernasconi C, Chiquet M. 1991. Tenascin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. Cell Reg 2:927–938.

Chiquet-Ehrismann R, Tannheimer M, Koch M, Brunner A, Spring J, Martin D, Baumgartner S, Chiquet M. 1994. Tenascin-C expression by fibroblasts is elevated in stressed collagen gels. J Cell Biol 127:2093–2101.

Chung CY, Erickson HP. 1994. Cell surface annexin II is a high affinity receptor for the alternatively spliced segment of tenascin-C. J Cell Biol 126:539–548.

Chung CY, Zardi L, Erickson HP. 1995. Binding of tenascin-C to soluble fibronectin and matrix fibrils. J Biol Chem 270:29012–29017.

Chung CY, Murphy-Ullrich JE, Erickson HP. 1996. Mitogenesis, cell migration, and loss of focal adhesions induced by tenascin-C interacting with its cell surface receptor, annexin II. Mol Biol Cell 7:883–892.

Chung CY, Erickson HP. 1997. Glycosaminoglycans modulate fibronectin matrix assembly and are essential for matrix incorporation of tenascin-C. J Cell Sci 110:1413–1419.

Cifuentes-Diaz C, Velasco E, Meunier FA, Goudou D, Belkadi L, Faille L, Murawsky M, Angaut-Petit D, Molgo J, Schachner M, Saga Y, Aizawa S, Rieger F. 1998. The peripheral nerve and the neuromuscular junction are affected in the tenascin-C-deficient mouse. Cell Mol Biol 44:357–379.

Cleek RL, Rege AA, Denner LA, Eskin SG, Mikos AG. 1997. Inhibition of smooth muscle cell growth in vitro by an antisense oligodeoxynucleotide released from poly(DL-lactic-co-glycolic acid) microparticles. J Biomed Mater Res 35:525–530.

Conway JF, Parry DA. 1991. Three-stranded alpha-fibrous proteins: the heptad repeat and its implications for structure. Int J Biol Macromol 13:14–16.

Cooke RM, Wilkinson AJ, Baron M, Pastore A, Tappin MJ, Campbell ID, Gregory H, Sheard B. 1987. The solution structure of human epidermal growth factor. Nature 327:339–341.

Copertino DW, Jenkinson S, Jones FS, Edelman GM. 1995. Structural and functional similarities between the promoters for mouse tenascin and chicken cytotactin. Proc Natl Acad Sci USA 92:2131–2135.

Copertino DW, Edelman GM, Jones FS. 1997. Multiple promoter elements differentially regulate the expression of the mouse tenascin gene. Proc Natl Acad Sci USA 93:1846–1851.

Costa AM, Peyrol S, Porto LC, Comparin JP, Foyatier JL, Desmouliere A. 1999. Mechanical forces induce scar remodeling: study in non-pressure-treated versus pressure-treated hypertrophic scars. Am J Pathol 155:1671–1679.

Cowan KN, Jones PL, Rabinovitch M. 1999. Regression of hypertrophied rat pulmonary arteries in organ culture is associated with suppression of proteolytic activity, inhibition of tenascin-C, and smooth muscle cell apoptosis. Circ Res 84:1223–1233.

Cowan KN, Jones PL, Rabinovitch M. 2000. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression of vascular disease. J Clin Invest 105: 21–34.

Crossin KL, Hoffman S, Grumet M, Thiery JP, Edelman GM. 1986. Site-restricted expression of cytotactin during development of the chicken embryo. J Cell Biol 102:1917–1930.

Crossin KL. 1991. Cytotactin binding: inhibition of stimulated proliferation and intracellular alkalinization in fibroblasts. Proc Natl Acad Sci USA 88:11403–11407.

Crossin KL. 1994. The functional role of cytotactin/tenascin in morphogenesis: a modest proposal. Perspect Dev Neurobiol 2:21–32.

Cserjesi P, Lilly B, Bryson L, Wang Y, Sassoon DA, Olson EN. 1992. MHox: a mesodermally restricted homeodomain protein that binds an essential site in the muscle creatine kinase enhancer. Development 115:1087–1101.

Cserjesi P, Lilly B, Hinkley C, Perry M, Olson EN. 1994. Homeodomain protein MHox and MADS protein myocyte enhancer-binding factor-2 converge on a common element in the muscle creatine kinase enhancer. J Biol Chem 269:16740–16745.

Cunha GR, Bigsby RM, Cooke PS, Sugimura Y. 1985. Stromal-epithelial interactions in adult organs. Cell Differ 17:137–148.

Curran T, Franza BR. 1988. Fos and Jun: the Ap-1 connection. Cell 55:395–397.

D'Alessandri L, Ranscht B, Winterhalter KH, Vaughan L. 1995. Contactin/F11 and tenascin-C co-expression in the chick retina correlates with formation of the synaptic plexiform layers. Curr Eye Res 14:911–926.

Davis GE. 1992. Affinity of integrins for damaged extracellular matrix: alpha v beta 3 binds to denatured collagen type I through RGD sites. Biochem Biophys Res Commun 182:1025–1031.

de Jong R, van der Heijden J, Meijlink F. 1993. DNA-binding specificity of the S8 homeodomain. Nucleic Acids Res 21:4711–4720.

Debbas M, White E. 1993. Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. Genes Dev 7:546–554.

Denda S, Muller U, Crossin KL, Erickson HP, Reichardt LF. 1998. Utilization of a soluble integrin-alkaline phosphatase chimera to characterize integrin alpha 8 beta 1 receptor interactions with tenascin: murine alpha 8 beta 1 binds to the RGD site in tenascin-C

fragments, but not to native tenascin-C. Biochemistry 37:5464–5474.
Derr LB, Chiquet-Ehrismann R, Gandour-Edwards R, Spence J, Tucker RP. 1997. The expression of tenascin-C with the AD1 variable repeat in embryonic tissues, cell lines and tumors in various vertebrate species. Differentiation 62:71–82.
Dodd J, Morton SB, Karagogeos D, Yamamoto M, Jessell TM. 1988. Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. Neuron 1:105–116.
Doolittle RF. 1984. Fibrinogen and fibrin. Annu Rev Biochem 53:195–229.
Dorries U, Schachner M. 1994. Tenascin mRNA isoforms in the developing mouse brain. J Neurosci Res 37:336–347.
Edelman GM, Jones FS. 1998. Gene regulation of cell adhesion: a key step in neural morphogenesis. Brain Res Rev 26:337–352.
Ekblom P, Aufderheide E. 1989. Stimulation of tenascin expression in mesenchyme by epithelial- mesenchymal interactions. Int J Dev Biol 33:71–79.
Ekblom M, Faissler R, Tomasini-Johansson B, Nilsson K, Ekblom P. 1993. Downregulation of tenascin expression by glucocorticoids in bone marrow stromal cells and in fibroblasts. J Cell Biol 123:1037–1045.
Elefteriou F, Exposito JY, Garrone R, Lethias C. 1997. Characterization of the bovine tenascin-X. J Biol Chem 272:22866–22874.
Elefteriou F, Exposito JY, Garrone R, Lethias C. 1999. Cell adhesion to tenascin-X mapping of cell adhesion sites and identification of integrin receptors. Eur J Biochem 263:840–848.
End P, Panayotou G, Entwistle A, Waterfield MD, Chiquet M. 1992. Tenascin: a modulator of cell growth. Eur J Biochem 209:1041–1051.
Erickson HP, Iglesias JL. 1984. A six-armed oligomer isolated from cell surface fibronectin preparations. Nature 311:267–269.
Erickson HP, Bourdon MA. 1989. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. Annu Rev Cell Biol 5:71–92.
Erickson HP. 1993. Gene knockouts of c-src, transforming growth factor B1, and tenascin suggest superfluous, nonfunctional expression of proteins. J Cell Biol 120:1079–1081.
Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC. 1992. Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119–128.
Ferguson JE, Schor AM, Howell A, Ferguson MW. 1990. Tenascin distribution in the normal human breast is altered during the menstrual cycle and in carcinoma. Differentiation 42:199–207.
Fischer D, Chiquet-Ehrismann R, Bernasconi C, Chiquet M. 1995. A single heparin binding region within the fibrinogen-like domain is functional in chick tenascin-C. J Biol Chem 270:3378–3384.
Fischer D, Brown-Ludi M, Schulthess T, Chiquet-Ehrismann R. 1997. Concerted action of tenascin-C domains in cell adhesion, anti-adhesion and promotion of neurite outgrowth. J Cell Sci 110:1513–1522.
Folkman J, Moscona A. 1978. Role of cell shape in growth control. Nature 273:345–349.
Forsberg E, Hirsch E, Frohlich L, Meyer M, Ekblom P, Aszodi A, Werner S, Fassler R. 1996. Skin wounds and severed nerves heal normally in mice lacking tenascin-C. Proc Natl Acad Sci USA 93:6594–6599.
Friedlander DR, Hoffman S, Edelman GM. 1988. Functional mapping of cytotactin: proteolytic fragments active in cell-substrate adhesion. J Cell Biol. 107:2329–2340.
Fukamauchi F, Mataga N, Wang YJ, Sato S, Youshiki A, Kusakabe M. 1996. Abnormal behavior and neurotransmissions of tenascin gene knockout mouse. Biochem Biophys Res Commun 221:151–156.
Fukamauchi F, Kusakabe M. 1997. Preprotachykinin A and cholecystokinin mRNAs in tenascin-gene knockout mouse brain. Neuropeptides 31:199–201.
Fukamauchi F, Mataga N, Wang YJ, Sato S, Yoshiki A, Kusakabe M. 1997. Tyrosine hydroxylase activity and its mRNA level in dopaminergic neurons of tenascin gene knockout mouse. Biochem Biophys Res Commun 231:356–359.
Fukamauchi F, Aihara O, Kusakabe M. 1998. Reduced mRNA expression of neuropeptide Y in the limbic system of tenascin gene disrupted mouse brain. Neuropeptides 32:265–268.
Fuss B, Pott U, Fischer P, Schwab ME, Schachner M. 1991. Identification of a cDNA clone specific for the oligodendrocyte-derived repulsive extracellular matrix molecule J1-160/180. J Neurosci Res 29:299–307.
Gates AT, Jones FS, Oakey RJ, Jones PL. 1999. Regulation of the pro-proliferative extracellular matrix protein tenascin-C by prx-1 & prx-2: a role for paired-related genes in vascular pathobiology. Circulation 100:3969–3970.
Geffrotin C, Garrido JJ, Tremet L, Vaiman M. 1995. Distinct tissue distribution in pigs of tenascin-X and tenascin-C transcripts. Eur J Biochem 231:83–92.
Gherzi R, Carnemolla B, Siri A, Ponassi M, Balza E, Zardi L. 1995a. Human tenascin gene: structure of the 5′-region, identification, and characterization of the transcription regulatory sequences. J Biol Chem 270:3429–3434.
Gherzi R, Ponassi M, Gaggero B, Zardi L. 1995b. The first untranslated exon of the human tenascin-C gene plays a regulatory role in gene transcription. FEBS Lett 369:335–339.
Gherzi R, Briata P, Boncinelli E, Ponassi M, Querze G, Viti F, Corte G, Zardi L. 1997. The human homeodomain protein OTX2 binds to the human tenascin-C promoter and trans-represses its activity in transfected cells. DNA Cell Biol 16:559–567.
Gherzi R, Leprini A, Siri A, Zardi L. 1998. Structure of 5′ region of human tenascin-R gene and characterization of its promoter. DNA Cell Biol 17:275–282.
Giese A, Loo MA, Norman SA, Treasurywala S, Berens ME. 1996. Contrasting migratory response of astrocytoma cells to tenascin mediated by different integrins. J Cell Sci 109:2161–2168.
Gooch KJ, Tennant CJ. 1997. Mechanical forces: their effects on cells and tissues. Berlin: Springer/Verlag.
Gotz B, Scholze A, Clement A, Joester A, Schutte K, Wigger F, Frank R, Spiess E, Ekblom P, Faissner A. 1996. Tenascin-C contains distinct adhesive, anti-adhesive, and neurite outgrowth promoting sites for neurons. J Cell Biol 132:681–699.
Gotz M, Bolz J, Joester A, Faissner A. 1997. Tenascin-C synthesis and influence on axonal growth during rat cortical development. Eur J Neurosci 9:496–506.
Gould VE, Koukoulis GK, Virtanen I. 1990. Extracellular matrix proteins and their receptors in the normal, hyperplastic and neoplastic breast. Cell Differ Dev 32:409–416.
Grobstein C. 1967. Mechanisms of organogenetic tissue interaction. Natl Cancer Inst Monogr 26:279–299.
Grumet M, Hoffman S, Crossin KL, Edelman GM. 1985. Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. Proc Natl Acad Sci USA 82:8075–8079.
Grumet M, Milev P, Sakurai T, Karthikeyan L, Bourdon M, Margolis RK, Margolis RU. 1994. Interactions with tenascin and differential effects on cell adhesion of neurocan and phosphacan, two major chondroitin sulfate proteoglycans of nervous tissue. J Biol Chem 269:12142–12146.
Gulcher JR, Nies DE, Marton LS, Stefansson K. 1989. An alternatively spliced region of the human hexabrachion contains a repeat of potential N-glycosylation sites. Proc Natl Acad Sci USA 86:1588–1592.
Gulcher JR, Nies DE, Alexakos MJ, Ravikant NA, Sturgill ME, Marton LS, Stefansson K. 1991. Structure of the human hexabrachion (tenascin) gene. Proc Natl Acad Sci USA 88:9438–9442.
Gundersen D, Tran-Thang C, Sordat B, Mourali F, Ruegg C. 1997. Plasmin-induced proteolysis of tenascin-C: modulation by T lymphocyte-derived urokinase-type plasminogen activator and effect on T lymphocyte adhesion, activation, and cell clustering. J Immunol 158:1051–1060.
Hagihara K, Miura R, Kosaki R, Berglund E, Ranscht B, Yamaguchi Y. 1999. Immunohistochemical evidence for the brevican-tenascin-R interaction: colocalization in perineuronal nets suggests a physiological role for the interaction in the adult rat brain. J Comp Neurol 410:256–264.
Hagios C, Koch M, Spring J, Chiquet M, Chiquet-Ehrismann R. 1996. Tenascin-Y: a protein of novel domain structure is secreted by differentiated fibroblasts of muscle connective tissue. J Cell Biol 134:1499–1512.

Hagios C, Brown-Luedi M, Chiquet-Ehrismann R. 1999. Tenascin-Y, a component of distinctive connective tissues, supports muscle cell growth. Exp Cell Res 253:607–617.

Handford PA, Baron M, Mayhew M, Willis A, Beesley T, Brownlee GG, Campbell ID. 1990. The first EGF domain from human factor IX contains a high affinity calcium binding site. EMBO J 9:475–480.

Hasegawa K, Yoshida T, Matsumoto K, Katsuta K, Waga S, Sakakura T. 1997. Differential expression of tenascin-C and tenascin-X in human astrocytomas. Acta Neuropathol (Berl) 93:431–437.

Hauzenberger D, Olivier P, Gundersen D, Ruegg C. 1999. Tenascin-C inhibits beta1 integrin-dependent T lymphocyte adhesion to fibronectin through the binding of its fnIII 1-5 repeats to fibronectin. Eur J Immunol 29:1435–1447.

Hedin U, Bottger BA, Forsberg E, Johansson S, Thyberg J. 1988. Diverse effects of fibronectin and laminin on phenotypic properties of cultured arterial smooth muscle cells. J Cell Biol 107:307–319.

Hedin U, Holm J, Hansson GK. 1991. Induction of tenascin in rat arterial injury: relationship to altered smooth muscle cell phenotype. Am J Pathol 139:649–656.

Hoffman S, Edelman GM. 1987. A proteoglycan with HNK-1 antigenic determinants is a neuron-associated ligand for cytotactin. Proc Natl Acad Sci USA 84:2523–2527.

Hoffman S, Crossin KL, Edelman GM. 1988. Molecular forms, binding functions, and developmental expression patterns of cytotactin and cytotactin-binding proteoglycan, an interactive pair of extracellular matrix molecules. J Cell Biol 106:519–532.

Houston P, Dickson MC, Ludbrook V, White B, Schwachtgen JL, McVey JH, Mackman N, Reese JM, Gorman DG. Campbell C, Braddock M. 1999. Fluid shear stress induction of the tissue factor promoter in vitro and in vivo is mediated by Egr-1. Arterioscler Thromb Vasc Biol 19:281–289.

Howeedy AA, Virtanen I, Laitinen L, Gould NS, Koukoulis GK, Gould VE. 1990. Differential distribution of tenascin in the normal, hyperplastic, and neoplastic breast. Lab Invest 63:798–806.

Howlett AR, Bailey N, Damsky C, Petersen OW, Bissell MJ. 1995. Cellular growth and survival are mediated by beta 1 integrins in normal human breast epithelium but not in breast carcinoma. J Cell Sci 108:1945–1957.

Hu Y, Flanagan J, Brennan DP, Zhou H, Ng KW, Eisman JA, Morrison NA. 1995. rHox: a homeobox gene expressed in osteoblastic cells. J Cell Biochem 59:486–497.

Hurley WL. 1989. Mammary gland function during involution. J Dairy Sci 72:1637–1646.

Ikuta T, Sogawa N, Ariga H, Ikemura T, Matsumoto K. 1998. Structural analysis of mouse tenascin-X: evolutionary aspects of reduplication of FNIII repeats in the tenascin gene family. Gene 217:1–13.

Imai K, Kusakabe M, Sakakura T, Nakanishi I, Okada Y. 1994. Susceptibility of tenascin to degradation by matrix metalloproteinases and serine proteinases. FEBS Lett 352:216–218.

Inaguma Y, Kusakabe M, Mackie EJ, Pearson CA, Chiquet-Ehrismann R, Sakakura T. 1988. Epithelial induction of stromal tenascin in the mouse mammary gland: from embryogenesis to carcinogenesis. Dev Biol 128:245–255.

Jaatinen T, Ruuskanen O, Truedsson L, Lokki ML. 1999. Homozygous deletion of the CYP21A-TNXA-RP2-C4B gene region conferring C4B deficiency associated with recurrent respiratory infections. Hum Immunol 60:707–714.

Joester A, Faissner A. 1999. Evidence for combinatorial variability of tenascin-C isoforms and developmental regulation in the mouse central nervous system. J Biol Chem 274:17144–17151.

Jones FS, Burgoon MP, Hoffman S, Crossin KL, Cunningham BA, Edelman GM. 1988. A cDNA clone for cytotactin contains sequences similar to epidermal growth factor-like repeats and segments of fibronectin and fibrinogen. Proc Natl Acad Sci USA 85:2186–2190.

Jones FS, Hoffman S, Cunningham BA, Edelman GM. 1989. A detailed structural model of cytotactin: protein homologies, alternative RNA splicing, and binding regions. Proc Natl Acad Sci USA 86:1905–1909.

Jones FS, Crossin KL, Cunningham BA, Edelman GM. 1990. Identification and characterization of the promoter for the cytotactin gene. Proc Natl Acad Sci USA 87:6497–6501.

Jones FS. 1991. The molecular biology of the extracellular matrix glycoprotein cytotactin: primary structure, alternative splicing, and characterization of the promoter. Ph.D. Thesis, The Rockefeller University, New York, NY.

Jones FS, Chalepakis G, Gruss P, Edelman GM. 1992. Activation of the cytotactin promoter by the homeobox-containing gene Evx-1. Proc Natl Acad Sci USA 89:2091–2095.

Jones PL, Schmidhauser C, Bissell MJ. 1993. Regulation of gene expression and cell function by extracellular matrix. Crit Rev Eukaryot Gene Exp 3:137–154.

Jones PL, Boudreau N, Myers CA, Erickson HP, Bissell MJ. 1995. Tenascin-C inhibits extracellular matrix-dependent gene expression in mammary epithelial cells: localization of active regions using recombinant tenascin fragments. J Cell Sci 108:519–527.

Jones PL, Rabinovitch M. 1996. Tenascin-C is induced with progressive pulmonary vascular disease in rats and is functionally related to increased smooth muscle cell proliferation. Circ Res 79:1131–1142.

Jones PL, Cowan KN, Rabinovitch M. 1997a. Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. Am J Pathol 150:1349–13460.

Jones PL, Crack J, Rabinovitch M. 1997b. Regulation of tenascin-C, a vascular smooth muscle cell survival factor that interacts with the alpha v beta 3 integrin to promote epidermal growth factor receptor phosphorylation and growth. J Cell Biol 139:279–293.

Jones PL, Jones FS, Zhou B, Rabinovitch M. 1999a. Induction of vascular smooth muscle cell tenascin-C gene expression by denatured type I collagen is dependent upon a beta3 integrin-mediated mitogen-activated protein kinase pathway and a 122-base pair promoter element. J Cell Sci 112:435–445.

Jones PL, Mohler ER, Schoen F, Levy RJ. 1999b. Tenascin-C & matrix metalloproteinase-2 in aortic valve disease. Circulation 100:2863–2864.

Jones PL, Morabito L, Baldwin HS, Raff G, Vitvitsky E, Spray TL, Gaynor JW. 1999c. Extracellular matrix remodeling with increased pulmonary blood flow: a potential role for Egr-1 in the transcriptional control of tenascin-C. Circulation 100:3970–3971.

Jrvinen TA, Jozsa L, Kannus P, Jrvinen TL, Kvist M, Hurme T, Isola J, Kalimo H, Jrvinen M. 1999. Mechanical loading regulates tenascin-C expression in the osteotendinous junction. J Cell Sci 112: 3157–3166.

Julian J, Chiquet-Ehrismann R, Erickson HP, Carson DD. 1994. Tenascin is induced at implantation sites in the mouse uterus and interferes with epithelial cell adhesion. Development 120:661–671.

Kammerer RA, Schulthess T, Landwehr R, Lustig A, Fischer D, Engel J. 1998. Tenascin-C hexabrachion assembly is a sequential two-step process initiated by coiled-coil alpha-helices. J Biol Chem 273: 10602–10608.

Kaplony A, Zimmermann DR, Fischer RW, Imhof BA, Odermatt BF, Winterhalter KH, Vaughan L. 1991. Tenascin Mr 220,000 isoform expression correlates with corneal cell migration. Development 112: 605–614.

Khachigian LM, Anderson KR, Halnon NJ, Gimbrone MA Jr, Resnick N, Collins T. 1997. Egr-1 is activated in endothelial cells exposed to fluid shear stress and interacts with a novel shear-stress-response element in the PDGF A- chain promoter. Arterioscler Thromb Vasc Biol 17:2280–2286.

Kheradmand F, Werner E, Tremble P, Symons M, Werb Z. 1998. Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. Science 280:898–902.

Kiernan BW, Garcion E, Ferguson J, Frost EE, Torres EM, Dunnett SB, Saga Y, Aizawa S, Faissner A, Kaur R, Franklin RJ, ffrench-Constant C. 1999. Myelination and behaviour of tenascin-C null transgenic mice. Eur J Neurosci 11:3082–3092.

Klein T, Arias AM. 1998. Interactions among delta, serrate and fringe modulate notch activity during Drosophila wing development. Development 125:2951–2962.

Koivunen E, Arap W, Valtanen H, Rainisalo A, Medina OP, Heikkila P, Kantor C, Gahmberg CG, Salo T, Konttinen YT, Sorsa T, Ruoslahti E, Pasqualini R. 1999. Tumor targeting with a selective gelatinase inhibitor. Nat Biotechnol 17:768–774.

Koukoulis GK, Virtanen I, Korhonen M. Laitinen L, Quaranta V, Gould VE. 1991. Immunohistochemical localization of integrins in the normal, hyperplastic, and neoplastic breast: correlations with their functions as receptors and cell adhesion molecules. Am J Pathol 139:787–799.

Kruse J, Keilhauer G, Faissner A, Timpl R, Schachner M. 1985. The J1 glycoprotein—a novel nervous system cell adhesion molecule of the L2/HNK-1 family. Nature 316:146–148.

Krushel LA, Prieto AL, Edelman GM, Crossin KL. 1994. Differential effects of cytotactin/tenascin fusion proteins on intracellular pH and cell morphology. J Cell Physiol 161:508–518.

Latijnhouwers MA, Bergers M, Van Bergen BH, Spruijt KI, Andriessen MP, Schalkwijk J. 1996. Tenascin expression during wound healing in human skin. J Pathol 178:30–35.

Leahy DJ, Hendrickson WA, Aukhil I, Erickson HP. 1992. Structure of a fibronectin type III domain from tenascin phased by MAD analysis of the selenomethionyl protein. Science 258:987–991.

Lendahl U. 1998. A growing family of Notch ligands. Bioessays 20: 103–107.

Leprini A, Gherzi R, Siri A, Querze G, Viti F, Zardi L. 1996. The human tenascin-R gene. J Biol Chem 271:31251–31254.

Lethias C, Descollonges Y, Boutillon MM, Garrone R. 1996. Flexilin: a new extracellular matrix glycoprotein localized on collagen fibrils. Matrix Biol 15:11–19.

Leussink B, Brouwer A, el Khattabi M, Poelmann RE. Gittenberger-de Groot AC, Meijlink F. 1995. Expression patterns of the paired-related homeobox genes MHox/Prx1 and S8/Prx2 suggest roles in development of the heart and the forebrain. Mech Dev 52:51–64.

Levine A, Bashan-Ahrend A, Budai-Hadrian O, Gartenberg D, Menasherow S, Wides R. 1994. Odd Oz: a novel Drosophila pair-rule gene. Cell 77:587–598.

Li Q, Jones PL. Gaynor JW, Spray T, Levy RJ. 1999. Tenascin-C upregulates thymosin beta4 and MMP-2 gene expression in human in human aortic valve cells. Mol Biol Cell 10:L51.

Lochter A, Schachner M. 1993. Tenascin and extracellular matrix glycoproteins: from promotion to polarization of neurite growth in vitro. J Neurosci 13:3986–4000.

Lu MF, Cheng HT, Kern MJ, Potter SS, Tran B, Diekwisch TG, Martin JF. 1999. prx-1 functions cooperatively with another paired-related homeobox gene, prx-2, to maintain cell fates within the craniofacial mesenchyme. Development 126:495–504.

Mackie EJ, Thesleff I, Chiquet-Ehrismann R. 1987. Tenascin is associated with chondrogenic and osteogenic differentiation in vivo and promotes chrondrogenesis in vitro. J Cell Biol 105:2569–2579.

Mackie EJ, Halfter W, Liverani D. 1988). Induction of tenascin in healing wounds. J Cell Biol 107:2757–2767.

Mackie EJ, Tucker RP. 1992. Tenascin in bone morphogenesis: expression by osteoblasts and cell type- specific expression of splice variants. J Cell Sci 103:765–771.

Mackie EJ, Ramsey S. 1996. Modulation of osteoblast behaviour by tenascin. J Cell Sci 109:1597–1604.

Mackie EJ, Tucker RP. 1999. The tenascin-C knockout revisited. J Cell Sci 112:3847–3853.

Malinda KM, Goldstein AL, Kleinman HK. 1997. Thymosin beta 4 stimulates directional migration of human umbilical vein endothelial cells. FASEB J 11:474–481.

Malinda KM, Sidhu GS, Mani H, Banaudha K, Maheshwari RK, Goldstein AL, Kleinman HK. 1999. Thymosin beta4 accelerates wound healing. J Invest Dermatol 113:364–368.

Marti A, Jehn B, Costello E, Keon N, Ke G, Martin F, Jaggi R. 1994. Protein kinase A and AP-1 (c-Fos/JunD) are induced during apoptosis of mouse mammary epithelial cells: oncogene 9:1213–1223.

Marti A, Lazar H, Ritter P, Jaggi R. 1999. Transcription factor activities and gene expression during mouse mammary gland involution. J Mammary Gland Biol Neoplasia 4:145–152.

Matsuda A, Tagawa Y, Yamamoto K, Matsuda H, Kusakabe M. 1999. Identification and immunohistochemical localization of annexin II in rat cornea. Curr Eye Res 19:368–375.

Matsumoto K, Saga Y, Ikemura T, Sakakura T, Chiquet-Ehrismann R. 1994. The distribution of tenascin-X is distinct and often reciprocal to that of tenascin-C. J Cell Biol 125:483–493.

Meiners S, Geller HM. 1997. Long and short splice variants of human tenascin differentially regulate neurite outgrowth. Mol Cell Neurosci 10:100–116.

Meiners S, Mercado ML, Nir-e-Kamal MS, Geller HM. 1999. Tenascin-C contains domains that independently regulate neurite outgrowth and neurite guidance. J. Neurosci 19:8443–8453.

Messent AJ, Tuckwell DS, Knauper V, Humphries MJ, Murphy G, Gavrilovic J. 1998. Effects of collagenase-cleavage of type I collagen on alpha2beta1 integrin-mediated cell adhesion. J Cell Sci 111: 1127–1135.

Mettouchi A, Cabon F, Montreau N, Dejong V, Vernier P, Gherzi R, Mercier G, Binetruy B. 1997. The c-Jun-induced transformation process involves complex regulation of tenascin-C expression. Mol Cell Biol 17:3202–3209.

Mieda M, Kikuchi Y, Hirate Y, Aoki M, Okamoto H. 1999. Compartmentalized expression of zebrafish ten-m3 and ten-m4, homologues of the drosophila ten(m)/odd Oz gene, in the central nervous system. Mech Dev 87:223–227.

Milev P, Friedlander DR, Sakurai T, Karthikeyan L, Flad M, Margolis RK, Grumet M, Margolis RU. 1994. Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. J Cell Biol 127:1703–1715.

Milev P, Maurel P, Haring M, Margolis RK, Margolis RU. 1996. TAG-1/axonin-1 is a high-affinity ligand of neurocan, phosphacan/protein-tyrosine phosphatase-zeta/beta, and N-CAM. J Biol Chem 271:15716–15723.

Milev P, Fischer D, Haring M, Schulthess T, Margolis RK, Chiquet-Ehrismann R, Margolis RU. 1997. The fibrinogen-like globe of tenascin-C mediates its interactions with neurocan and phosphacan/protein-tyrosine phosphatase-zeta/beta. J Biol Chem 272:15501–15509.

Milev P, Chiba A, Haring M, Rauvala H, Schachner M, Ranscht B, Margolis RK, Margolis RU. 1998. High affinity binding and overlapping localization of neurocan and phosphacan/protein-tyrosine phosphatase-zeta/beta with tenascin-R, amphoterin, and the heparin-binding growth-associated molecule. J Biol Chem 273:6998–7005.

Minet AD, Rubin BP, Tucker RP, Baumgartner S, Chiquet-Ehrismann R. 1999. Teneurin-1, a vertebrate homologue of the Drosophila pair-rule gene ten- m, is a neuronal protein with a novel type of heparin-binding domain. J Cell Sci 112:2019–2032.

Mitrovic N, Schachner M. 1995. Detection of tenascin-C in the nervous system of the tenascin-C mutant mouse. J Neurosci Res 42: 710–717.

Moch H, Torhorst J, Durmuller U, Feichter GE, Sauter G, Gudat F. 1993. Comparative analysis of the expression of tenascin and established prognostic factors in human breast cancer. Pathol Res Pract 189:510–514.

Morawietz H, Ma YH, Vives F, Wilson E, Sukhatme VP, Holtz J, Ives HE. 1999. Rapid induction and translocation of Egr-1 in response to mechanical strain in vascular smooth muscle cells. Circ Res 84: 678–687.

Morel Y, Bristow J, Gitelman SE, Miller WL. 1989. Transcript encoded on the opposite strand of the human steroid 21-hydroxylase/complement component C4 gene locus. Proc Natl Acad Sci USA 86:6582–6586.

Moscoso LM, Cremer H, Sanes JR. 1998. Organization and reorganization of neuromuscular junctions in mice lacking neural cell adhesion molecule, tenascin-C, or fibroblast growth factor-5. J Neurosci 18:1465–1477.

Murphy-Ullrich JE, Lightner VA, Aukhil I, Yan YZ, Erickson HP. 1991. Focal adhesion integrity is downregulated by the alternatively spliced domain of human tenascin. J Cell Biol 115:1127–1136.

Nakao N, Hiraiwa N, Yoshiki A, Ike F, Kusakabe M. 1998. Tenascin-C promotes healing of Habu-snake venom-induced glomerulonephritis: studies in knockout congenic mice and in culture. Am J Pathol 152:1237–1245.

Natali PG, Nicotra MR, Bartolazzi A, Cavaliere R, Bigotti A. 1993. Integrin expression in cutaneous malignant melanoma: association of the alpha 3/beta 1 heterodimer with tumor progression. Int J Cancer 54:68–72.

Neville M, Daniel C. 1987. The mammary gland: development, regulation and function. New York: Plenum.

Nicolo G, Salvi S, Oliveri G, Borsi L, Castellani P, Zardi L. 1990. Expression of tenascin and of the ED-B containing oncofetal fibronectin isoform in human cancer. Cell Differ Dev 32:401–408.

Nishi T, Weinstein J, Gillespie WM, Paulson JC. 1991. Complete primary structure of porcine tenascin: detection of tenascin transcripts in adult submaxillary glands. Eur J Biochem 202:643–648.

Norenberg U, Wille H, Wolff JM, Frank R, Rathjen FG. 1992. The chicken neural extracellular matrix molecule restrictin: similarity with EGF-, fibronectin type III-, and fibrinogen-like motifs. Neuron 8:849–863.

Norenberg U, Hubert M, Brummendorf T, Tarnok A, Rathjen FG. 1995. Characterization of functional domains of the tenascin-R (restrictin) polypeptide: cell attachment site, binding with F11, and enhancement of F11-mediated neurite outgrowth by tenascin-R. J Cell Biol 130:473–484.

Oberhauser AF, Marszalek PE, Erickson HP, Fernandez JM. 1998. The molecular elasticity of the extracellular matrix protein tenascin. Nature 393:181–185.

Ohta M, Sakai T, Saga Y, Aizawa S, Saito M. 1998. Suppression of hematopoietic activity in tenascin-C-deficient mice. Blood 91:4074–4083.

Oleszewski M, Beer S, Katich S, Geiger C, Zeller Y, Rauch U, Altevogt P. 1999. Integrin and neurocan binding to L1 involves distinct Ig domains. J Biol Chem 274:24602–24610.

Oohashi T, Zhou XH, Feng K, Richter B, Morgelin M, Perez MT, Su WD, Chiquet-Ehrismann R, Rauch U, Fassler R. 1999. Mouse ten-m/Odz is a new family of dimeric type II transmembrane proteins expressed in many tissues. J Cell Biol 145:563–577.

Paganelli G, Grana C, Chinol M, Cremonesi M, De Cicco C, De Braud F, Robertson C, Zurrida S, Casadio C, Zoboli S, Siccardi AG, Veronesi U. 1999. Antibody-guided three-step therapy for high grade glioma with yttrium- 90 biotin. Eur J Nucl Med 26:348–357.

Patthy L. 1990. Homology of a domain of the growth hormone/prolactin receptor family with type III modules of fibronectin. Cell 61:13–14.

Pearson CA, Pearson D, Shibahara S, Hofsteenge J, Chiquet-Ehrismann R. 1988. Tenascin: cDNA cloning and induction by TGF-beta. EMBO J 7:2977–2982.

Perides G, Erickson HP, Rahemtulla F, Bignami A. 1993. Colocalization of tenascin with versican, a hyaluronate-binding chondroitin sulfate proteoglycan. Anat Embryol (Berl) 188:467–479.

Pesheva P, Probstmeier R, Skubitz AP, McCarthy JB. Furcht LT, Schachner M. 1994. Tenascin-R (J1 160/180) inhibits fibronectin-mediated cell adhesion—functional relatedness to tenascin-C. J Cell Sci 107:2323–2333.

Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ. 1992. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells [published erratum appears in Proc Natl Acad Sci USA 1993 Mar 15;90(6):2556]. Proc Natl Acad Sci USA 89:9064–9068.

Phillips GR, Krushel LA, Crossin KL. 1998. Domains of tenascin involved in glioma migration. J Cell Sci 111:1095–1104.

Prieto AL, Jones FS, Cunningham BA, Crossin KL, Edelman GM. 1990. Localization during development of alternatively spliced forms of cytotactin mRNA by in situ hybridization. J Cell Biol 111:685–698.

Prieto AL, Andersson-Fisone C, Crossin KL. 1992. Characterization of multiple adhesive and counteradhesive domains in the extracellular matrix protein cytotactin. J Cell Biol 119:663–678.

Prieto AL, Edelman GM, Crossin, KL. 1993. Multiple integrins mediate cell attachment to cytotactin/tenascin. Proc Natl Acad Sci USA 90:10154–10158.

Probstmeier R, Pesheva P. 1999. Tenascin-C inhibits beta1 integrin-dependent cell adhesion and neurite outgrowth on fibronectin by a disialoganglioside-mediated signaling mechanism. Glycobiology 9:101–114.

Rathjen FG, Wolff JM, Chiquet-Ehrismann R. 1991. Restrictin: a chick neural extracellular matrix protein involved in cell attachment co-purifies with the cell recognition molecule F11. Development 113:151–164.

Rauch U, Clement A, Retzler C, Frohlich L, Fassler R, Gohring W, Faissner A. 1997. Mapping of a defined neurocan binding site to distinct domains of tenascin-C. J Biol Chem 272:26905–26912.

Renshaw MW, Price LS. Schwartz MA. 1999. Focal adhesion kinase mediates the integrin signaling requirement for growth factor activation of MAP kinase. J Cell Biol 147:611–618.

Rettig WJ, Triche TJ, Garin-Chesa P. 1989. Stimulation of human neuronectin secretion by brain-derived growth factors. Brain Res 487:171–177.

Rettig WJ, Erickson HP, Albino AP, Carin-Chesa P. 1994. Induction of human tenascin (neuronectin) by growth factors and cytokines: cell type-specific signals and signalling pathways. J Cell Sci 107:487–497.

Retzler C, Gohring W, Rauch U. 1996. Analysis of neurocan structures interacting with the neural cell adhesion molecule N-CAM. J Biol Chem 271:27304–27310.

Riva P, Franceschi G, Frattarelli M, Riva N, Guiducci G, Cremonini AM, Giuliani G, Casi M, Gentile R, Jekunen AA, Kairemo KJ. 1999. 131I radioconjugated antibodies for the locoregional radioimmunotherapy of high-grade malignant glioma—phase I and II study. Acta Oncol 38:351–359.

Roovers K, Davey G, Zhu X, Bottazzi ME, Assoian RK. 1999. Alpha5beta1 integrin controls cyclin D1 expression by sustaining mitogen-activated protein kinase activity in growth factor-treated cells. Mol Biol Cell 10:3197–3204.

Roskelley CD, Desprez PY, Bissell MJ. 1994. Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. Proc Natl Acad Sci USA 91:12378–12382.

Rupert KL, Rennebohm RM, Yu CY. 1999. An unequal crossover between the RCCX modules of the human MHC leading to the presence of a CYP21B gene and a tenascin TNXB/TNXA-RP2 recombinant between C4A and C4B genes in a patient with juvenile rheumatoid arthritis. Exp Clin Immunogenet 16:81–97.

Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S. 1992. Mice develop normally without tenascin. Genes Dev 6:1821–1831.

Sakakura T, Sakagami Y, Nishizuka Y. 1979. Persistence of responsiveness of adult mouse mammary gland to induction by embryonic mesenchyme. Dev Biol 72:201–210.

Sakakura T. 1991. New aspects of stroma-parenchyma relations in mammary gland differentiation. Int Rev Cytol 125:165–202.

Salmivirta M, Elenius K, Vainio S, Hofer U, Chiquet-Ehrismann R, Thesleff I, Jalkanen M. 1991. Syndecan from embryonic tooth mesenchyme binds tenascin. J Biol Chem 266:7733–7739.

Schenk S, Chiquet-Ehrismann R. 1994. Tenascins. In: Ruoslahti E, Engvall E, editors. Methods in Enzymology, Volume 245. New York: Academic Press. p 52–61.

Schenk S, Chiquet-Ehrismann R, Battegay EJ. 1999. The fibrinogen globe of tenascin-C promotes basic fibroblast growth factor-induced endothelial cell elongation. Mol Biol Cell 10:2933–2943.

Schmidhauser C, Bissell MJ, Myers CA, Casperson GF. 1990. Extracellular matrix and hormones transcriptionally regulate bovine beta-casein 5′ sequences in stably transfected mouse mammary cells. Proc Natl Acad Sci USA 87:9118–9122.

Schmidhauser C, Casperson GF, Myers CA, Sanzo KT, Bolten S, Bissell MJ. 1992. A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix-dependent regulation of β-casein gene expression. Mol Biol Cell 3:699–709.

Schnapp LM, Hatch N, Ramos DM, Klimanskaya IV, Sheppard D, Pytela R. 1995. The human integrin alpha 8 beta 1 functions as a receptor for tenascin, fibronectin, and vitronectin. J Biol Chem 270:23196–23202.

Schwachtgen JL, Houston P, Campbell C, Sukhatme V, Braddock M. 1998. Fluid shear stress activation of egr-1 transcription in cultured human endothelial and epithelial cells is mediated via the extracellular signal-related kinase 1/2 mitogen-activated protein kinase pathway. J Clin Invest 101:2540–2549.

Seiffert M, Beck SC, Schermutzki F, Muller CA, Erickson HP, Klein G. 1998. Mitogenic and adhesive effects of tenascin-C on human

hematopoietic cells are mediated by various functional domains. Matrix Biol 17:47–63.
Settles DL, Kusakabe M, Steindler DA, Fillmore H, Erickson HP. 1997. Tenascin-C knockout mouse has no detectable tenascin-C protein. J Neurosci Res 47:109–117.
Siri A, Knauper V, Veirana N, Caocci F, Murphy G, Zardi L. 1995. Different susceptibility of small and large human tenascin-C isoforms to degradation by matrix metalloproteinases. J Biol Chem 270:8650–8654.
Sonderegger P, Kunz S, Rader C, Buchstaller A, Berger P, Vogt L, Kozlov SV, Ziegler U, Kunz B, Fitzli D, Stoeckli ET. 1998. Discrete clusters of axonin-1 and NgCAM at neuronal contact sites: facts and speculations on the regulation of axonal fasciculation. Prog Brain Res 117:93–104.
Speek M, Barry F, Miller WL. 1996. Alternate promoters and alternate splicing of human tenascin-X, a gene with 5′ and 3′ ends buried in other genes. Hum Mol Genet 5:1749–1758.
Spring J, Beck K, Chiquet-Ehrismann R. 1989. Two contrary functions of tenascin: dissection of the active sites by recombinant tenascin fragments. Cell 59:325–334.
Sriramarao P, Bourdon MA. 1993. A novel tenascin type III repeat is part of a complex of tenascin mRNA alternative splices. Nucleic Acids Res 21:163–168.
Strange R, Li F, Saurer S, Burkhardt A, Friis RR. 1992. Apoptotic cell death and tissue remodelling during mouse mammary gland involution. Development 115:49–58.
Streuli CH, Bailey N, Bissell MJ. 1991. Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. J Cell Biol 115:1385–1395.
Streuli CH, Schmidhauser C, Kobrin M, Bissell MJ, Derynck R. 1993. Extracellular matrix regulates expression of the TGF-beta 1 gene. J Cell Biol 120:253–260.
Streuli C. 1999. Extracellular matrix remodelling and cellular differentiation. Curr Opin Cell Biol 11:634–640.
Suzuki M, Naruse K, Asano Y, Okamoto T, Nishikimi N, Sakurai T, Nimura Y, Sokabe M. 1997. Up-regulation of integrin beta 3 expression by cyclic stretch in human umbilical endothelial cells. Biochem Biophys Res Commun 239:372–376.
Sympson CJ, Talhouk RS, Alexander CM, Chin JR, Clift SM, Bissell MJ, Werb Z. 1994. Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression [published erratum appears in J Cell Biol 1996 Feb;132(4):following 752]. J Cell Biol 125:681–693.
Talhouk RS, Chin JR, Unemori EN, Werb Z, Bissell MJ. 1991. Proteinases of the mammary gland: developmental regulation in vivo and vectorial secretion in culture. Development 112:439–449.
Talhouk RS, Bissell MJ, Werb Z. 1992. Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. J Cell Biol 118:1271–1282.
Talts JF, Wirl G, Dictor M, Muller WJ, Fassler R. 1999. Tenascin-C modulates tumor stroma and monocyte/macrophage recruitment but not tumor growth or metastasis in a mouse strain with spontaneous mammary cancer. J Cell Sci 112:1855–1864.
Tan S-S, Crossin KL, Hoffman S, Edelman GM. 1987. Asymmetric expression in somites of cytotactin and its proteoglycan ligand is correlated with neural crest cell distribution. Proc Natl Acad Sci USA 84:7977–7981.
Tanaka Y, Schuster DP, Davis EC, Patterson GA, Botney MD. 1996. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. J Clin Invest 98:434–442.
Thesleff IE, Mackie E, Vainio S, Chiquet-Ehrismann R. 1987. Changes in the distribution of tenascin during tooth development. Development 101:289–296.
Thomasset N, Lochter A, Sympson CJ, Lund LR, Williams DR, Behrendtsen O, Werb Z, Bissell MJ. 1998. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. Am J Pathol 153:457–467.
Tremble P, Chiquet-Ehrismann R, Werb Z. 1994. The extracellular matrix ligands fibronectin and tenascin collaborate in regulating collagenase gene expression in fibroblasts. Mol Biol Cell 5:439–453.
Tucker RP. 1991. The sequential expression of tenascin mRNA in epithelium and mesenchyme during feather morphogenesis. Roux's Arch Dev Biol 200:108–112.
Tucker RP, Hagios C, Chiquet-Ehrismann R. 1999. Tenascin-Y in the developing and adult avian nervous system. Dev Neurosci 21:126–133.
Tuckwell DS, Ayad S, Grant ME, Takigawa M, Humphries MJ. 1994. Conformation dependence of integrin-type II collagen binding: inability of collagen peptides to support alpha 2 beta 1 binding, and mediation of adhesion to denatured collagen by a novel alpha 5 beta 1-fibronectin bridge. J Cell Sci 107:993–1005.
Tuffy R, Kretsinger RH. 1975: Troponin and paralbumin calcium binding regions predicted in myosin light chain and lysozyme. Science 187:167–169.
Vainio S, Jalkanen M, Thesleff I. 1989; Syndecan and tenascin expression is induced by epithelial-mesenchymal interactions in embryonic tooth mesenchyme. J Cell Biol 108:1945–1953.
Vaughan L, Weber P, D'Alessandri L, Zisch AH, Winterhalter KH. 1994. Tenascin-contactin/F11 interactions: a clue for a developmental role? Perspect Dev Neurobiol 2:43–52.
Volkmer H, Zacharias U, Norenberg U, Rathjen FG. 1998. Dissection of complex molecular interactions of neurofascin with axonin-1, F11, and tenascin-R, which promote attachment and neurite formation of tectal cells. J Cell Biol 142:1083–1093.
Vollmer G, Michna H, Ebert K, Knuppen R. 1992. Down-regulation of tenascin expression by antiprogestins during terminal differentiation of rat mammary tumors. Cancer Res 52:4642–4648.
Wallner K, Li C, Fishbein MC, Shah PK, Sharifi BG. 1999. Arterialization of human vein grafts is associated with tenascin-C expression. J Am Coll Cardiol 34:871–875.
Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ. 1997. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. J Cell Biol 137:231–245.
Webb CM, Zaman G, Mosley JR, Tucker RP, Lanyon LE, Mackie EJ. 1997. Expression of tenascin-C in bones responding to mechanical load. J Bone Miner Res 12:52–58.
Weber P, Zimmermann DR, Winterhalter KH, Vaughan L. 1995. Tenascin-C binds heparin by its fibronectin type III domain five. J Biol Chem 270:4619–4623.
Weber P, Ferber P, Fischer R, Winterhalter KH, Vaughan L. 1996. Binding of contactin/F11 to the fibronectin type III domains 5 and 6 of tenascin is inhibited by heparin. FEBS Lett 389:304–308.
Weber P, Montag D, Schachner M, Bernhardt RR. 1998. Zebrafish tenascin-W, a new member of the tenascin family. J Neurobiol 35:1–16.
Weber P, Bartsch U, Rasband MN, Czaniera R, Lang Y, Bluethmann H, Margolis RU, Levinson SR, Shrager P, Montag D, Schachner M. 1999. Mice deficient for tenascin-R display alterations of the extracellular matrix and decreased axonal conduction velocities in the CNS. J Neurosci 19:4245–4262.
Wehrle-Haller B, Chiquet M. 1993. Dual function of tenascin: simultaneous promotion of neurite growth and inhibition of glial migration. J Cell Sci 106:597–610.
Weller A, Beck S, Ekblom P. 1991. Amino acid sequence of mouse tenascin and differential expression of two tenascin isoforms during embryogenesis. J Cell Biol 112:355–362.
Whitby DJ, Ferguson MW. 1991. The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development 112:651–668.
Wung BS, Cheng JJ, Chao YJ, Hsieh HJ, Wang DL. 1999. Modulation of Ras/Raf/extracellular signal-regulated kinase pathway by reactive oxygen species is involved in cyclic strain-induced early growth response-1 gene expression in endothelial cells. Circ Res 84:804–812.
Xiao ZC, Tayler J, Montag D, Rougon G, Schachner M. 1996. Distinct effects of recombinant tenascin-R in neuronal cell functions and identification of the domain interacting with neuronal recognition molecule F3/11. Euro J Neurosci 8:766–782.

Xiao ZC, Hillenbrand R, Schachner M, Thermes S, Rougon G, Gomez S. 1997. Signaling events following the interaction of the neuronal adhesion molecule F3 with the N-terminal domain of tenascin-R. J Neurosci Res 49:698–709.

Xiao ZC, Ragsdale DS, Malhotra JD, Mattei LN, Braun PE, Schachner M, Isom LL. 1999. Tenascin-R is a functional modulator of sodium channel beta subunits. J Biol Chem 274:26511–26517.

Xu X, Doolittle RF. 1990. Presence of a vertebrate fibrinogen-like sequence in an echinoderm. Proc Natl Acad Sci USA 87:2097–2101.

Yamamoto K, Dang QN, Kennedy SP, Osathanondh R, Kelly RA, Lee RT. 1999. Induction of tenascin-C in cardiac myocytes by mechanical deformation. J Biol Chem 274:21840–21846.

Yang H, Xiao ZC, Becker B, Hillenbrand R, Rougon G, Schachner M. 1999. Role for myelin-associated glycoprotein as a functional tenascin-R receptor. J Neurosci Res 55:687–701.

Yang Z, Mendoza AR, Welch TR, Zipf WB, Yu CY. 1999. Modular variations of the human major histocompatibility complex class III genes for serine/threonine kinase RP, complement component C4, steroid 21-hydroxylase CYP21, and tenascin TNX (the RCCX module): a mechanism for gene deletions and disease associations. J Biol Chem 274:12147–12156.

Yano Y, Geibel J, Sumpio BE. 1997. Cyclic strain induces reorganization of integrin alpha 5 beta 1 and alpha 2 beta 1 in human umbilical vein endothelial cells. J Cell Biochem 64:505–513.

Yokosaki Y, Monis H, Chen J, Sheppard D. 1996. Differential effects of the integrins alpha9beta1, alphavbeta3, and alphavbeta6 on cell proliferative responses to tenascin: roles of the beta subunit extracellular and cytoplasmic domains. J Biol Chem 271:24144–24150.

Yuasa S. 1996. Bergmann glial development in the mouse cerebellum as revealed by tenascin expression. Anat Embryol (Berl) 194:223–234.

Zacharias U, Norenberg U, Rathjen FG. 1999. Functional interactions of the immunoglobulin superfamily member F11 are differentially regulated by the extracellular matrix proteins tenascin-R and tenascin-C. J Biol Chem 274:24357–24365.

Zagzag D, Friedlander DR, Dosik J, Chikramane S, Chan W, Greco MA, Allen JC, Dorovini-Zis K, Grumet M. 1996. Tenascin-C expression by angiogenic vessels in human astrocytomas and by human brain endothelial cells in vitro. Cancer Res 56:182–189.

Zempo N, Kenagy RD, Au YP, Bendeck M, Clowes MM, Reidy MA, Clowes AW. 1994. Matrix metalloproteinases of vascular wall cells are increased in balloon-injured rat carotid artery. J Vasc Surg 20:209–217.

Zhao Y, Young SL. 1995. TGF-beta regulates expression of tenascin alternative-splicing isoforms in fetal rat lung. Am J Physiol 268: L173–L180.

Zhu X, Assoian RK. 1995. Integrin-dependent activation of MAP kinase: a link to shape-dependent cell proliferation. Mol Biol Cell 6:273–282.

Zisch AH. D'Alessandri L, Ranscht B, Falchetto R, Winterhalter KH, Vaughan L. 1992. Neuronal cell adhesion molecule contactin/F11 binds to tenascin via its immunoglobulin-like domains. J Cell Biol 119:203–213.