Structure, Physiology, and Biochemistry of Collagens

2

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Abstract

Tendons and ligaments are connective tissues that guide motion, share loads, and transmit forces in a manner that is unique to each as well as the anatomical site and biomechanical stresses to which they are subjected. Collagens are the major molecular components of both tendons and ligaments. The hierarchical structure of tendon and its functional properties are determined by the collagens present, as well as their supramolecular organization. There are 28 different types of collagen that assemble into a variety of supramolecular structures. The assembly of specific supramolecular structures is dependent on the interaction with other matrix molecules as well as the cellular elements. Multiple suprastructural assemblies are integrated to form the functional tendon/ligament. This chapter begins with a discussion of collagen molecules. This is followed by a definition of the supramolecular structures assembled by different collagen types. The general principles involved in the assembly of collagen-containing suprastructures are presented focusing on the regulation of tendon collagen fibrillogenesis. Finally, site-specific differences are discussed. While generalizations can be made, differences exist between different tendons as well as between tendons and ligaments. Compositional differences will impact structure that in turn will determine functional differences. Elucidation of the unique physiology and pathophysiology of different tendons and ligaments will require an appreciation of the role compositional differences have on collagen suprastructural assembly, tissue organization, and function.

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2.1 Introduction

The composition and structure of tendons and ligaments play important roles in their functions. Tendons attach muscles to bones, and ligaments act to connect bone to bone across a joint space. Both tendons and ligaments are fibrous connective tissues that are composed of cells within an extracellular matrix rich in collagens, proteoglycans, and water. However, the distinct function of each connective tissue is intricately linked to its specific composition and resulting structure. This also can be described for tendon type or location (e.g., axial versus limb, flexor versus extensor) and for regions of tendons (e.g., midsubstance versus enthesis). The composition of tendons and ligaments allows these connective tissues to help guide motion, to resist abnormal displacement of bones and center the actions of several muscles, and to share load and distribute force. The general compositions of tendons and ligaments are similar. Both are composed of water (50–60 % for tendons and 60–70 % for ligaments), collagens (70–80 % dry weight for tendons and more than 80 % for ligaments), and proteoglycans, including small leucine-rich proteoglycans [1]. The collagens present and the supramolecular structures assembled play significant roles in the function of these musculoskeletal connective tissues. Tendons are hierarchical structures with structure and function being closely linked (Fig. 2.1). Tendons are composed primarily of collagen fibrils. Bundles of fibrils are organized as fibers. Fibers are grouped together with tenocytes as fascicles, which are surrounded by a cellular, loose connective tissue, the endotenon. The outer edge of a bundle of fascicles comprising the tendon is demarcated by a contiguous epitenon cover. Structure and composition of such collagen-rich tissues allow for tendons and ligaments to be relatively compliant at low energy, low loading forces, yet to increase in stiffness with increasing forces and loads.

This chapter provides an overview of collagens with a focus on tendons and ligaments. The first section provides an overview of collagens focusing on those collagens found within tendons and ligaments. This is followed by a discussion of collagen synthesis, fibril assembly, as well as fibril growth, and maturation from the assembly of protofibrils to the organization of fibrils into mature fibers. Finally, the tissue structure and function of the collagen-rich extracellular matrix of connective tissues like tendons and ligaments are discussed in the context of differing roles for the collagens in musculoskeletal physiology.

2.2 Collagens

Collagens are proteins that are major components of the extracellular matrix of connective tissues. Members of the collagen family are trimers. They have at least one collagenous or COL domain as well as non-collagenous or NC domains. The number and structure of COL and NC domains are dependent upon the specific collagen type. The importance of these domains will be discussed. Among the genomes of vertebrates and higher invertebrates, there are 28 distinct collagen glycoproteins that are encoded by at least 45 genes. These collagens have been given Roman numeral designations (I–XXVIII) in chronological order of discovery, and they are classified by type based on domain structure and their supramolecular organization. For each collagen type,

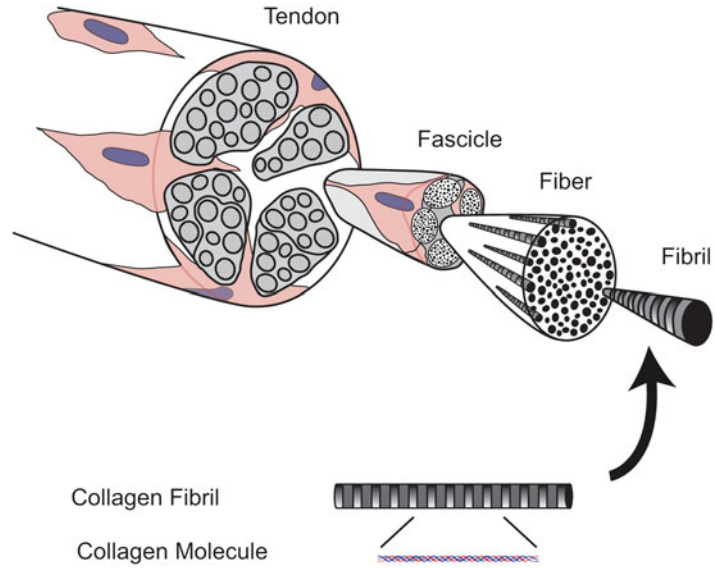

Fig. 2.1 Hierarchical structure of tendon. Tendons are hierarchical structures composed primarily of collagen fibrils. Triple helical collagen molecules assemble to form fibrils. Fibrils bundle together to form fibers. Within the mature tendon, fibers are grouped together with tenocytes within fascicles that are surrounded by a cellular, loose connective tissue, the endotenon. The outer edge of a bundle of fascicles is the epitenon; it contiguously covers the outside of the tendon

each genetically distinct alpha chain is designated by Arabic numerals. The alpha chains of one collagen type are unique from the alpha chains of another collagen type. Each distinct alpha chain is encoded by a different gene, and each distinct alpha chain has its own primary (domain) structure which contributes to the classification by collagen type. For example, the human α1(I) chain is encoded by the *COL1A1* gene and the mouse α1(II) chain by the *Col2a1* gene. Collagens can be homotrimeric; that is, they are composed of three identical alpha chains, like $[\alpha1(II)]_3$ for collagen II. However, collagens also can be heterotrimeric, comprised of alpha chains encoded by different genes of the same collagen type, like $[\alpha1(I)]$ $[\alpha2(I)]_2$ for collagen I. Moreover, it is possible for a single collagen type to have multiple chain compositions, like $[\alpha1(V)]$ $[\alpha2(V)]_2$, $[\alpha1(V)]$ $[\alpha2(V)]$ $[\alpha3(V)]$, or $[\alpha1(V)]_3$ for collagen V.

Collagens may be grouped into classes based upon their suprastructural organization (Table 2.1). Some collagens are fibril-forming; for example, collagens I, II, and III. These collagens form fibrils with a distinct 67 nm periodicity. There are also Fibril-Associated Collagens with Interrupted Triple helices (FACIT) collagens that associate with collagen fibrils and interact with collagenous and non-collagenous proteins. Collagens in each category have their own specialized function and contribute to higher order tissue structures. As collagens from various categories assemble together with varying abundance and with co-polymerization with other non-collagenous macromolecules, they contribute to a tissue suprastructure and thus to its function. Likewise, with development and growth as well as repair and remodeling, the relative abundance and localization of different collagenous and non-collagenous macromolecules are major determinants of the structure and function of that tissue. This is certainly true of tendons and ligaments.

In tendons and ligaments, greater than 90 % of the connective tissue is composed of collagen I [1]. While collagen I is the predominant collagen in these tissues, other collagen types and non-collagenous macromolecules add diversity to the matrix and regulate just how fibril and fiber assembly occurs. This ultimately leads to tissue-specific structure and organization. As is the case with tendons by type, location, and even by zone within each tendon, collagen-containing suprastructures function differently based upon their complex macromolecular compositions that include other collagen types as well as

Table 2.1 General classification of collagen types

Classification	Collagen types	Supramolecular structure
Fibril-forming collagen	I, II, III	Striated fibrils
	V, XI	Striated fibrils, retain N-terminal regulatory domains
	XXIV, XXVII	Unknown
FACIT[a] collagens	IX, XII, XIV	Associated with fibrils, other interactions
FACIT-like collagens	XVI, XIX, XXI, XXII	Interfacial regions, basement membrane zones
Network-forming collagens		
Basement membrane	IV	Chicken wire network with lateral association
Beaded filament-forming	VI	Beaded filaments, networks
Anchoring fibrils	VII	Laterally associated anti-parallel dimers
Hexagonal networks	VIII, X	Hexagonal lattices
Transmembrane collagens	XIII, XVII, XXIII, XXV	Transmembrane and shed soluble ecto-domains
	Gliomedins, ectodysplasin	
Multiplexin collagens (Endostatin–XV and -XVIII)	XV, XVIII	Basement membranes, cleaved C-terminal domains influence angiogenesis
Other molecules with collagenous domains	XXVI, XXVIII	Collagenous domains in primarily non-collagenous molecules
	Acetylcholinesterase, adiponectin, C1q, collectins, surfactant protein, others	

[a]Fibril-associated collagen with interrupted triple helix

non-collagenous components. These additional macromolecules may be substantial or only occur in minute quantities. However, typically the architecture and function of tendons and ligaments are determined by the composite structure of collagen suprastructures. In the following section, the collagens contributing to the suprastructural organization of tendons and ligaments will be reviewed.

2.3 Fibril-Forming Collagens

The fibril-forming collagen subfamily includes collagens I, II, III, V, XI, XXIV and XXVII. Collagens I, II, III, V, and XI have been found in tendons and in ligaments [1–3]. These collagens have a long uninterrupted triple helical domain (ca. 300 nm). Fibril-forming collagen genes cluster into three distinct subclasses[4] and this carries over into functional subclasses. Collagens I, II and III are the most abundant proteins in the vertebrate body and are the bulk components of all collagen fibrils. Within tendons and ligaments, type III collagen is found in greater abundance during embryonic development; however, with maturation levels of collagen III decrease, though in the chicken it still persists in the endotenon and tendon sheath [5]. Moreover, in mature tendons and ligaments of rabbits, it has been noted that collagen III comprises 5 % and 10 %, respectively, of all collagen content [6]. Otherwise, collagen I is the predominant collagen of the tendon and ligament mid-substance. It should be noted that collagen II is generally found within the fibrocartilaginous zone of the enthesis site for both tendons and ligament [7, 8]. Collagens V and XI are quantitatively minor collagens found co-assembled with types I, II and III; they are found on the surfaceome (i.e., plasma membrane and pericellular matrix) of tendon fibroblasts [9]. This fibril-forming subclass retains portions of the N-terminal propeptide and is involved in the regulation of fibril assembly.[2] Collagens XXIV and XXVII make up the third subclass and have differences relative to the other fibril-forming collagen types including, shorter helical regions that are interrupted. Their structural organization

and specific roles remain to be elucidated, and they have yet to be found within tendons and ligaments.

The fibril-forming collagens are synthesized and secreted as procollagens. Procollagens contain a non-collagenous C-terminal propeptide and an N-terminal propeptide. The N-propeptide is composed of several non-collagenous domains and a short collagenous domain. The presence of the propeptides prevents premature assembly of collagen molecules into fibrils. The initial assembly of collagen into fibrils is regulated by the processing of the propeptides which involves several enzymes. The C-propeptides are processed by bone morphogenetic protein 1(BMP-1)/tolloid proteinases or furin [10, 11]. The processing of the N-propeptides involves certain members of the a-disintegrin-and-metalloproteinase-with-thrombospondin-like-motifs family (ADAMTS 2, 3 and 14) as well as BMP-1 [12]. Propeptide processing enzymes have specificity for each different collagen type.[10, 12] Propeptide processing may be complete, thus leaving a collagen molecule with one large central triple helical domain and terminal, short non-collagenous sequences termed the telopeptides, as is the case for collagens I and II. However, with collagens III, V, and XI, processing can be incomplete, with the retention of a C-telopeptide and a partially processed N-propeptide domain, which have been implicated in the regulation of fibrillogenesis [13–17]. After propeptide processing, collagen molecules self-assemble to form striated fibrils with a periodicity of 67 nm. Within each fibril, collagen molecules arrange longitudinally in staggered arrays. Thus, a gap occurs between the ends of neighboring molecules, and this gap-overlap structure is present in all collagen fibrils with a 67 nm D-periodic banding pattern. This is presented schematically in Fig. 2.2a.

Collagen fibrils are heterotypic. That is, they are assembled from mixtures of two or more fibril-forming collagen types. In tendons and ligaments, collagens I and III are the quantitatively major fibril-forming collagens with collagen II present within fibrocartilaginous regions. Heterotypic collagen fibrils of tendons and ligaments also contain quantitatively minor amounts of collagens V and XI; as mentioned above, these regulatory fibril-forming collagens are characterized by partial processing of N-propeptide domains. The N-propeptides have a flexible or hinge domain (NC2) between the triple helical domain (COL1) and a short triple helical domain (COL2). The N-terminal domain (NC3) is composed of a variable domain and a proline/arginine-rich protein (PARP) domain. Partial processing removes the PARP yet retains the hinge, COL2, and variable domains [18–20]. The regulatory fibril-forming collagens co-assemble with the major fibril-forming collagens in the heterotypic fibril; however, the N-terminal domain of the regulatory fibril forming collagens cannot be integrated into the staggered packing of the helical domains. The rigid COL2 domain of the collagen V or XII molecule can project toward the fibril surface in the gap region of the assembled fibril (Fig. 2.2b). Recent findings have demonstrated that alteration in collagens V and/or XI affects tendon fibril assembly during tendon development. Changes include altered fibril structure, decreased fibril number and abnormal fibril and fiber organization [21]. Thus, interactions between the fibrillar collagens affect the organization of collagen fibrils within collagen-rich tissues like tendons and ligaments [22].

2.4 Fibril-Associated Collagens with Interrupted Triple Helices (FACIT)

FACIT collagens closely interact with fibril-forming collagens. These molecules affect the surface properties of fibrils as well as fibril packing. Collagens IX, XII, XIV and XX are FACIT collagens. Type IX collagen is primarily found interacting with collagen II. Collagen IX is also a proteoglycan with covalently attached glycosaminoglycan side chains in cartilage [23]. This is also true with Type XII collagen [24]. Collagens XII and XIV have been found throughout musculoskeletal connective tissues, including tendons and ligaments at various times during development; [25, 26] collagen XIV has been found specifically at the bone-ligament interface in bovine entheses [27] In general, FACIT collagens have short COL

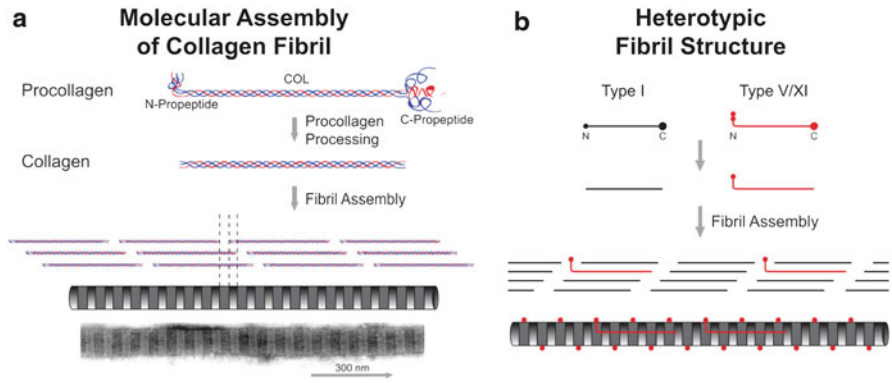

Fig. 2.2 Fibril-forming collagens. (**a**) Fibril-forming collagens are synthesized as procollagens. Procollagens contain a central COL domain and flanking propeptide N-and C-terminal NC domains. Propeptides are processed and the resulting collagen molecules assemble to form striated fibrils. Each fibrillar collagen molecule is approximately 300 nm (4.4D) in length and 1.5 nm in diameter. Within the fibril, the collagen molecules are staggered N to C in a pattern that gives rise to the D-periodic repeat. At the bottom of the panel, a D-periodic collagen fibril from tendon is presented. The characteristic alternating light and dark pattern represents the respective overlap and gap regions of the fibril. (**b**) Collagen fibrils are heterotypic. That is, they are co-assembled from quantitatively major fibril-forming collagens (e.g., I, II, or III) and regulatory fibril-forming collagens (V or XI). Regulatory fibril-forming collagens have a partially processed N-terminal propeptide, retaining a non-collagenous domain that must be in/on the gap region/fibril surface. The heterotypic interaction is involved in nucleation of fibril assembly (This figure has been adapted from Birk and Bruckner [131])

domains interrupted by NC domains with an N-terminal NC domain that projects into the interfibrillar space (Fig. 2.3). FACIT collagens have two C-terminal domains NC1 and COL1 that are believed to interact with the collagen I fibrils. At the N-termini of FACIT collagens, the large globular NC domains protrude from the fibril surface [2]. FACIT collagens along the surface of fibrils have been shown affect fiber suprastructures and tendon biomechanics [25, 26].

The FACIT-like collagens have features in common with FACIT collagen, though they are structurally and functionally unique. This FACIT-like group includes collagens XVI, XIX, XXI and XXII, yet their roles in musculoskeletal connective tissues have yet to be elucidated [28–32].

2.5 Basement Membrane Collagen

Collagen IV is considered a basement membrane collagen. It is the collagenous component of an integrated network of several matrix molecules that form an extracellular matrix that defines the interface between tissues [33, 34], including to some extent musculoskeletal tissues, particularly those that are adjacent to or fed by vasculature. For example, vasculature is found along the sheath, paratenon, and epitenon of tendons in a tendon-specific manner and basically along the "epiligament," or surrounding surface layer of tissue for ligaments [1]. Throughout the body, there are diverse networks of basement membranes composed of many macromolecules that are anatomical site-dependent. Likewise, there are several subtypes of collagen IV which are composed of different stoichiometries of 6 collagen IV-encoding genes COL4A1 through COL4A6 reviewed by Khoshnoodi et al. [35].

2.6 Beaded Filament-Forming Collagen

Collagen VI is ubiquitous within connective tissue; it is found as an extensive filamentous network with collagen fibrils, and is often enriched in pericellular regions. Collagen VI can be assembled into several different tissue forms,

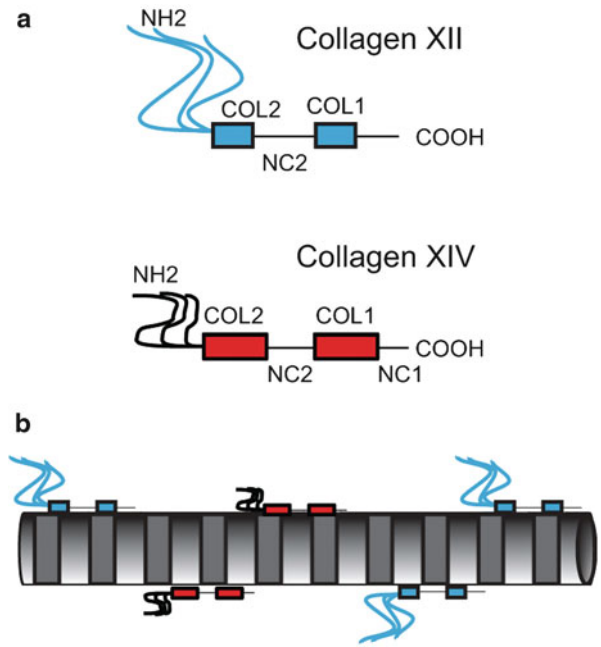

Fig. 2.3 FACIT collagens associate with fibrils. (**a**) The domain structures of FACIT collagens found in tendons and ligaments are illustrated. Note that all FACITs have alternative spliced variants, and collagen XII can have glycosaminoglycan chains attached covalently. The FACIT collagens have 2-3 COL domains and 3-4 NC domains with a large N-terminal NC domain that projects into the inter-fibrillar space. (**b**) The FACIT collagens all associate with the surface of collagen fibrils, including N-truncated isoforms due to alternative splicing in collagen XII. Collagen XII is capable of other non-fibril interactions (not shown) (This figure has been adapted from Birk and Bruckner [131])

including beaded microfibrils, broad banded structures and hexagonal networks [36–38]. Collagen VI interacts with many extracellular molecules including: collagens I, II, IV, XIV; microfibril-associated glycoprotein (MAGP-1); perlecan; decorin and biglycan; hyaluronan, heparin and fibronectin, as well as integrins and the cell-surface proteoglycan NG2. Based on the tissue-localization and large number of potential interactions, collagen VI has been proposed to integrate different components of the extracellular matrix, including cells [39]. Collagen VI also may influence cell proliferation, apoptosis, migration, and differentiation. Thus, collagen VI is involved in the development of tissue-specific extracellular matrices, repair processes and in the maintenance of tissue homeostasis. In musculoskeletal tissue, collagen VI has proven to be essential; mutations have been shown to cause various forms of muscular dystrophy as well as proximal joint contractures involving tendons in humans[40–43] (**See also Chap. 12 by Bushby**). In tendons, when collagen VI is removed via null *Col6a1* mouse model, tenocyte expression changes due to a lack of cell-matrix interactions [42]. In tendons, the absence of collagen VI results in increases in fibril density, significant reductions in load and stiffness, increased matrix metalloproteinase activity, and overall dysfunctional regulation of fibrillogenesis [42].

Collagen VI is commonly formed as a heterotrimer composed of α1(VI), α2(VI) and α3(VI) chains [39, 44]. Each monomer has a 105 nm triple helical domain with flanking N- and C-terminal globular domains. The α3(VI) chain of the heterotrimer can be processed extracellularly. In addition, structural heterogeneity is introduced by alternative splicing of domains, primarily of the α3(VI) N–terminal domain. Three additional α chains of type VI collagen have been described, α4(VI), α5(VI), α6(VI); these chains have high homology with the α3(VI) chain and may form

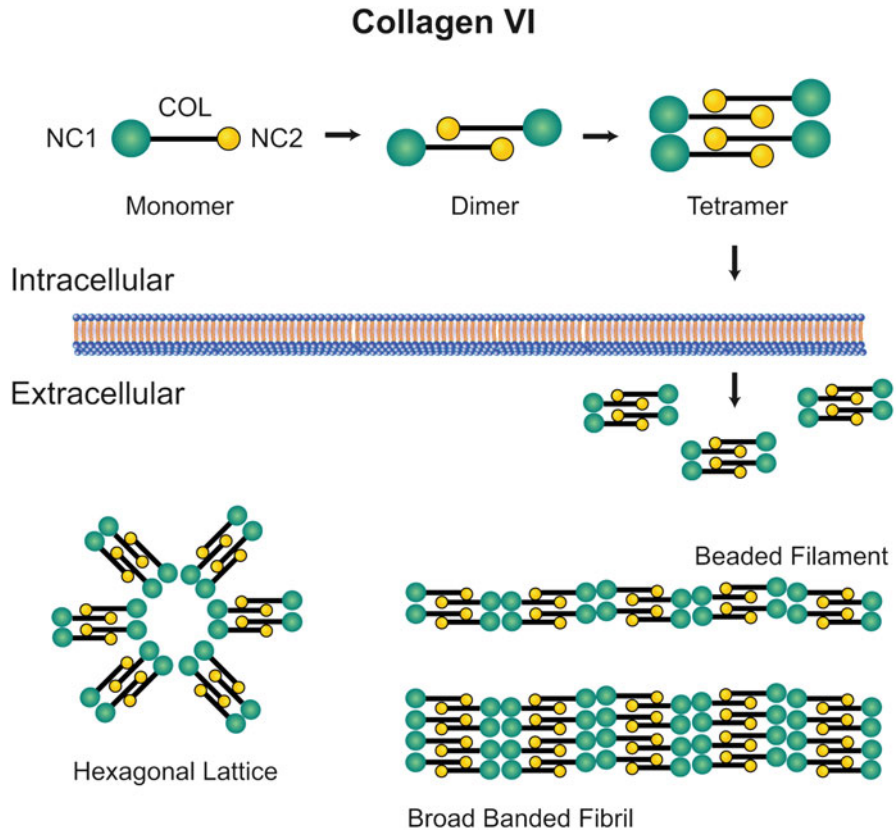

Fig. 2.4 Assembly of collagen VI suprastructures. Collagen VI monomers have a C-terminal NC domain, a central triple helical domain, and an N-terminal NC domain. The monomers assemble N-C to form dimers. Tetramers assemble from two dimers aligned in-register. The tetramers are secreted and form the building blocks of 3 different collagen VI suprastructures: beaded filaments, broad banded fibrils and hexagonal lattices. These suprastructures form via end-to-end interactions of tetramers and varying degrees of lateral association (This figure has been adapted from Birk and Bruckner [131])

additional isoforms [45, 46]. The supramolecular assembly of collagen VI begins intracellularly (Fig. 2.4). Two collagen VI monomers assemble in a lateral, anti-parallel fashion to form a dimer; the monomers are staggered by 30 nm with the C-terminal domains interacting with the helical domains. The resulting overlap generates a central 75 nm helical domain flanked by a non-overlapped region with the N– and C-globular domains, each about 30 nm. The C-terminal domain-helical domain interactions are stabilized by disulfide bonds near the ends of each overlapped region [47]. The overlapped helices form into a supercoil of the two monomers in the central region [48]. Two dimers then align to form, tetramers, also intracellularly. The tetramers are secreted and associate end-to-end to form beaded filaments extracellularly. The newly formed thin, beaded filaments (3–10 nm) have a periodicity of approximately 100 nm; they laterally associate to form beaded microfibrils [36], and they are found in hexagonal lattices [49]. The broad banded fibrils represent continued lateral growth of beaded microfibrils and/or lateral association of preformed beaded microfibrils. In contrast, hexagonal lattices are formed via end-to-end interactions of tetramers in a non-linear fashion [49].

Like fibrillar collagen structures, collagen VI-rich supramolecular aggregates are composite structures with other integrated molecules that

modulate the functional properties of the ultimate suprastructure. For example, biglycan interactions with the collagen VI tetramer induced formation of hexagonal lattices, instead of beaded microfibrils; though, decorin, which binds to the same site, was less effective in inducing hexagonal lattice formation [49]. Thus, analogous to fibril formation, the interaction of small leucine-rich proteoglycans with collagen VI can influence the structure of the tissue aggregate and therefore its function. The interaction of collagens with such molecules in a multitude of ways allows for the assembly of different suprastructures in adjacent regions or tissues with different functions, even sometimes as simply as with the addition or removal of non-collagenous molecules.

2.7 Network-Forming Collagens

Collagens VIII and X are closely related short chain collagens, with comparable gene and protein structures [39, 50]. While collagen VIII is not typically found in musculoskeletal tissues, collagen X has a very restricted distribution, found only in hypertrophic cartilage. This collagen is a homotrimer composed of α1(X) chains and the supramolecular form is a hexagonal lattice [51]. Collagen X was recently reviewed [52].

2.8 Transmembrane Collagens

Transmembrane collagens include collagens XIII, XVII, XXIII, and XXV. They are all homotrimers and contain an N-terminal cytoplasmic domain and a large C-terminal domain containing multiple COL domains with NC interruptions providing flexibility. Transmembrane collagens are so classified because they have a hydrophobic membrane spanning domain. Between this domain and an adjacent extracellular linker domain is the first COL domain involved in trimerization which is also subject to proteolytic cleavage generating a shed extracellular domain. Of the transmembrane collagens, collagen XIII is found in musculoskeletal tissues, particularly in myotendinous and neuromuscular junctions [53, 54].

2.9 Procollagen Synthesis, Collagen Fibril Assembly, Growth and Maturation

Collagen synthesis, assembly, and maturation require a sequence of well controlled intracellular and extracellular events. Collagen genes are transcribed from DNA into mRNA within the nucleus. Transcripts are transported out of the nucleus and translated into procollagen monomers, which are post-translationally modified in the rough endoplasmic reticulum prior to assembly into procollagen triple helices. The extent of these modifications is affected by the rate of triple helix formation, which is in turn affected by the primary structure of the alpha chain propeptides. For example, point mutations in the Gly-X-Y sequence may result in altered molecular properties for that chain as well as dysfunctional regulation of chain selection, helix formation, or post-translational modification. These molecules are then secreted as procollagens, which prevents premature molecular assembly into suprastructures. Procollagens are then processed extracellularly, in most cases, and require collagen type-specific metalloproteinases. Processing may, however, begin during the transport of newly synthesized procollagens to the cell surface [55, 56]. Processed collagen triples helices are then cross-linked. The relationship between protein structure and triple helix assembly and collagen fibril formation will be discussed below, as well as the growth and maturation of fibrils in tendons and ligaments.

2.10 Triple Helix Assembly and the Impact of Primary Structure on Secondary, Tertiary, and Quaternary Structures

After collagen pre-pro-peptides have been translated, they are directed into the lumen of the rough endoplasmic reticulum (RER). Modifications in the pre-pro-peptide include cleavage of the N-terminal signal for transport to the RER so that

it becomes a propeptide, or a pro-alpha chain [57]. Pro-alpha chains undergo hydroxylation of prolyl and lysyl residues followed by glycosylation of hydroxylysyl residues. Three pro-alpha chains will trimerize; this involves the selection and alignment of appropriate alpha chains with subsequent assembly into specific trimeric collagen molecules [58]. Trimerization is initiated through interactions among the non-collagenous trimerization domains of alpha chains at their C-termini in the RER [57].

The primary amino acid sequence of collagen alpha chains affects the protein structure at all other levels. The primary structure of the collagen alpha chain features a COL domain that will coil into a left-handed helix lacking intra-chain hydrogen bonds. Three alpha chains will supercoil to form a triple helix. This parallel, right-handed superhelix is stabilized by inter-chain hydrogen bonds that are almost perpendicular to the triple helical axis. Because of the imino acid content, collagen polypeptides assume an elongated polyproline II-like helix with all peptide bonds in the *trans* configuration. The pitch of the polyproline II helix in collagenous polypeptides corresponds to three amino acids, almost exactly. Steric constraints require that only glycine, the smallest amino acid, can occupy the positions at the center of the triple helix. Thus, the triple helical domains have a repeating $(Gly\text{-}X\text{-}Y)_n$ structure with the X and Y positions being any of the 21 proteinogenic amino acids of the universal genetic code. However, the X and Y positions are typically occupied by proline and hydroxyproline, which are necessary for helix formation and stability, respectively. Replacement of the glycine residues with other amino acids interrupts the triple helix motif and causes the rod-like structures to have rigid kinks or flexible hinges [59, 60]. This occurs in many collagen types with primary structures containing more than one helical domain thus providing flexibility, e.g., in collagen V. However, the substitution of glycine residues also may be the result of missense mutations in collagen genes and manifest as the underlying etiology for mild or severe, systemic connective tissue diseases [61, 62].

As post-translational modification and triple helix assembly occur concomitantly, once initiated, trimerization must be controlled to allow for alpha chain post-translational events like hydroxylation and glycosylation to occur. After or as collagen alpha chains are translated, amino acids within the triple helical domains of each chain can be modified. Co-translational modification of these domains includes hydroxylation and glycosylation. Collagen polypeptides contain two unique amino acids, hydroxyproline and hydroxylysine, which are important downstream for triple helix stability and glycosylation, respectively. As the propeptides are synthesized, these unique amino acids are introduced by enzymatic hydroxylation of almost all prolyl- and some of the lysyl residues in the Y-positions. Hydroxyproline residues are important in triple helix stability. The 4-trans hydroxyl group assists in directing the free hydroxyproline, similar to that of Y-hydroxyproline in collagen triple helices. The forced integration of proline into Y-positions absorbs more ring deformation when compared with physiological hydroxylated collagen [63, 64]. Collagens are further modified post-translationally after secretion and during aggregate assembly.

Fibril structure also is affected by post-translational glycosylation of collagens. Covalent modifications occurring after polypeptide synthesis are important in collagens and have an impact on fibril assembly [65–67]. The circumference of collagen triple helical domains is affected by the extent of hydroxylation of lysyl residues and subsequent galactosylation and glucosyl-galactosylation of hydroxylysyl residues. Intermolecular center-to-center distances correlate with the extent of glycosylation, particularly if post-translational modifications affect those regions of polypeptides situated in overlap regions of the fibril. The extent of glycosylation of hydroxylysine modifications can be manipulated by features such as increased enzyme activity levels[68] and disease-causing mutations [62]. Rates of procollagen triple helix formation can be substantially reduced in the rough endoplasmic reticulum by collagen mutations. Over-modification leads to compromised fibrillar organization. Yet

different extents of glycosylation can serve as a physiological mode of regulation in normal native tissue, particularly when levels of post-translational modification are tissue-specific and thus contribute to the structural properties of tissues. Intrafibrillar water also has been shown to affect the molecular organization of collagen fibrils. As variable amounts of water are incorporated within the fibrils, intermolecular distances between lateral or longitudinal neighbors have been shown to differ [69, 70]. When collagen fibrils are dried, their D-periodicity shortens intermolecular lateral distances reduce.

Collagenous domains of alpha chains are distinctly rich in *cis*-peptide bonds due to their high content of imino acids that favor *cis*-peptide bond formation. Kinetically, a great deal of energy is required to cause *cis*-peptide bonds to comply with triple helix formation. Isomerization of each *cis*-peptide bond encountered is necessary during collagen triple helix formation which is a zipper-like process. Helix assembly thus involves a slow folding process when compared to other proteins [71, 72]. At the start of triple helix formation, a variable number of *cis* bonds are distributed throughout the still unfolded procollagen polypeptides. Hence, the folding times required for full-length triple helix formation are heterogeneous depending upon kinetic obstacles. In fibroblasts, the *cis* to *trans* isomerization of Gly-Pro-, but not X-Hyp peptide bonds, is catalyzed by cyclophilin B, which acts as peptidyl prolyl *cis/trans*-isomerase. Cyclophilin B can be inhibited by the immuno-suppressor cyclosporin A [73]. Moreover, cyclophilin B along with prolyl-3-hydroxylase and cartilage-associated protein (Crtap) form a ternary complex with high chaperone activity in the endoplasmic reticulum. Prolyl-3-hydroxylase introduces a single 3-Hyp-residue at the C-terminal end of the triple helical domain of nascent fibrillar procollagens. This ternary complex localizes cyclophilin B-activity to the initiation sites for procollagen folding. This allows for efficient catalytic isomerization of peptidyl-prolyl *cis* bonds. Such a role is supported by data from humans where null mutations in LEPRE1 and CRTAP, the genes encoding human prolyl-3-hydroxylase and Crtap, respectively, cause severe recessive osteogenesis imperfecta [74].

Additional modifications occur post-translati onally after secretion of procollagen and during supramolecular assembly. Extracellular lysyl oxidases can convert the amino groups on some of the hydroxylysine- and lysine residues in the collagen polypeptide chain to aldehydes that form aldols or β-ketoamines [57]. This happens when the aldehydes of hydoxylysine or lysine residues react with aldehydes or amino groups on lysines, respectively, in other chains to generate intra- and inter-molecular covalent crosslinks [75–77]. In some cases, cross-linking of collagen molecules at early stages of aggregation can modulate the suprastructural outcome of fibrillogenesis or the formation of networks.

2.11 Collagen Fibril Assembly

The assembly and deposition of collagen fibrils with tissue-specific structures and organizations involves a sequence of events that occur in both intracellular and extracellular compartments. Collagen molecules are synthesized, hydroxylated, glycosylated, assembled from three polypeptides, and folded in the rough endoplasmic reticulum. Then packaging of the trimers occurs in the Golgi, and transport is via specialized and elongated intracellular compartments with secretion at the cell surface. Collagen fibrils are composites of different matrix molecules, and control of heteropolymeric mixing and trimer type stoichiometry begins within the intracellular compartments. Moreover, secretion of different matrix molecules and modifying enzymes occurs with specific spatial, temporal, and circumstantial patterns. Therefore, the character of the assembled fibrillar matrix depends upon not only the collagen types synthesized, but on the regulated interactions with procollagen processing enzymes, fibril-associated molecules (e.g., proteoglycans and FACITs), and adhesive glycoproteins. The spatial and temporal regulation of mixing during packaging and transport or at the sites of secretion provides a mechanism whereby limited numbers of matrix molecules can be

assembled to produce the diversity of structure and function observed across tissues.

Extracellularly in the developing tendon, fibril assembly begins in deep recesses or channels defined by the fibroblast surface [55, 78–80]. In these micro-domains, protofibrils are assembled [78, 81]. These immature fibrils have small and uniform diameters as well as short lengths compared to mature fibrils. These extracellular channels have been shown to form at the time of secretion as specialized post-Golgi secretory compartments, fuse with the fibroblast membrane and are maintained due to slow membrane recycling associated with the presence of the assembled protofibril [78, 81]. Other data also suggest that intracellular processing of procollagen may occur within elongated Golgi-to-plasma membrane compartments (GPCs); this is followed by the extrusion of protofibrils through a cellular protrusion, where GPC fuse to fibroblast plasma membranes [55, 79]. Either way, protofibrils are ultimately present extracellularly. Once the protofibrils are deposited into the extracellular matrix, more compartmentalization occurs. That is, fibrils form small fibers and then larger structures characteristic of the specific tissue (e.g., large fibers in tendon). This compartmentalized hierarchy within the extracellular allows the fibroblast to exert control over the extracellular steps of matrix assembly (Fig. 2.5).

2.12 Assembly and Growth of Mature Tendon Collagen Fibrils

In mature tendon, collagen fibrils are functionally continuous. That is, fibrils are long, though lengths unmeasured, and have diameters ranging between 20 and 500 nm depending on the tissue and developmental stage [82–84]. However, during tendon development collagen fibrils are assembled near the fibroblast surface as uniform and relatively short D periodic protofibrils with diameters in the range of 20–40 nm, lengths of 4–12 μm, and tapered ends [81, 82, 85, 86]. The newly assembled protofibrils are deposited and incorporated into the developing tendon extracellular matrix as small bundles of fibrils or immature fibers. There they are stabilized via interactions with macromolecules such as FACITs and small leucine-rich proteoglycans (Fig. 2.6). Tendon maturation continues with linear fibril growth involving end overlap of the protofibrils, followed by lateral growth in tendons and ligaments. Lateral fibril growth features the association and fusion of fibrils laterally to generate larger diameter fibrils. This process involves molecular rearrangement of fibrils so that a cylindrical fibril structure is generated. To accomplish this, some or all of the collagen stabilizing components stabilizing are lost or replaced. Throughout this process, the number of intra- and intermolecular crosslinking plays an important role in regulation of structure turnover and stability. Increases in stability generated by crosslinking improve mechanical stability that varies by location within tendons and ligament and anatomically throughout the body. The specific roles of collagen accretion in fibril assembly/repair during regeneration remain to be elucidated. Moreover, contributions in tissue homeostasis and with normal turnover and repair are likely.

2.13 Regulation of Collagen Fibril Assembly and Growth

Regulation of collagen fibrillogenesis is tissue-specific. Within each tissue, interactions occur amongst many different classes of molecules, such as processing enzymes, heterotypic fibril-forming collagens, FACITs, and small leucine-rich proteoglycans (SLRPs) as well as other glycoproteins, such as, fibronectin, tenasin X, etc. Many regulatory interactions occur and one way to impose some order on the discussion is to consider three classes of interactions; molecules that act as organizers, nucleators, and regulators [87].

For example, significant increases in collagen fibril diameters occur during growth and development of tendon, and mechanical properties of the tendon are dependent on increases in fibril diameter seen.[88] Tendon fibroblasts express collagen I as the major fibril-forming collagen with minor

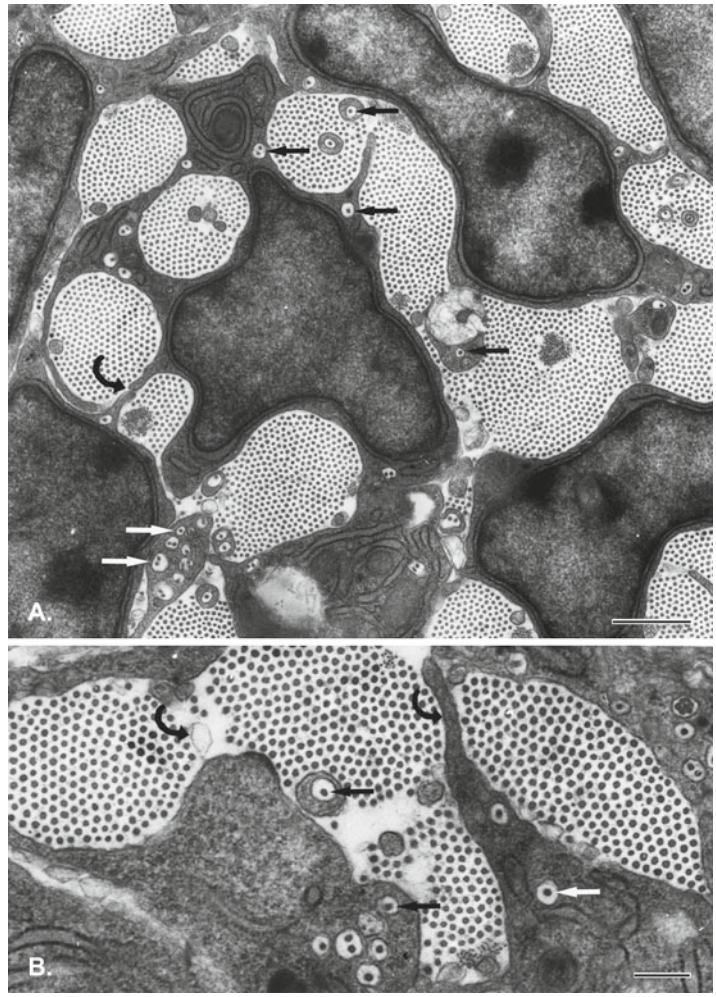

Fig. 2.5 Extracellular compartmentalization of fibril and fiber assembly by tenocytes. In the developing chicken tendon, extracellular compartmentalization of the different levels of matrix assembly is seen in both panels (**a**) and (**b**). These panels were generated from cross-sections of 14-day chicken embryo tendon cut perpendicular to the tendon axis A series of micro-domains are evident. Protofibrils are assembled in fibril-forming channels (*arrowheads*). Protofibrils are deposited into the developing matrix in fiber-forming spaces where fibers coalesce to form fibers. Fibers continue to become larger as a result of the aggregation of adjacent fibers in a third domain. As cytoplasmic processes that define fiber-forming compartments retract (*curved arrow*), fibers (fibril bundles) are allowed to coalesce into larger aggregates characteristic of mature tissue. Bar, 1 micrometer (Modified from Birk and Linsenmayer 1994)

quantities of collagen V. Thus, tendon fibrils are heterotypic, containing collagens I and V.

Collagens V and XI have been shown to nucleate collagen fibril formation in self-assembly assays [89–92]. The alpha-chains of collagens V and XI are highly homologous; thus, these collagens represent different isoforms of a single collagen type. A mouse model with a targeted deletion within the *Col5a1* gene is embryonic lethal due to a lack of fibril formation in the mesenchyme; though collagen I is synthesized and secreted, protofibrils are not properly assembled [91]. Similarly, in two separate mouse models where collagen XI is absent (cho/cho, ablated *Col11a1* alleles; *Col2a1*-null mice, from which the α3(XI) chain is derived), animals develop chondrodysplasia (cho) where cartilage is devoid of fibrils.[93–96] These data demonstrate a key

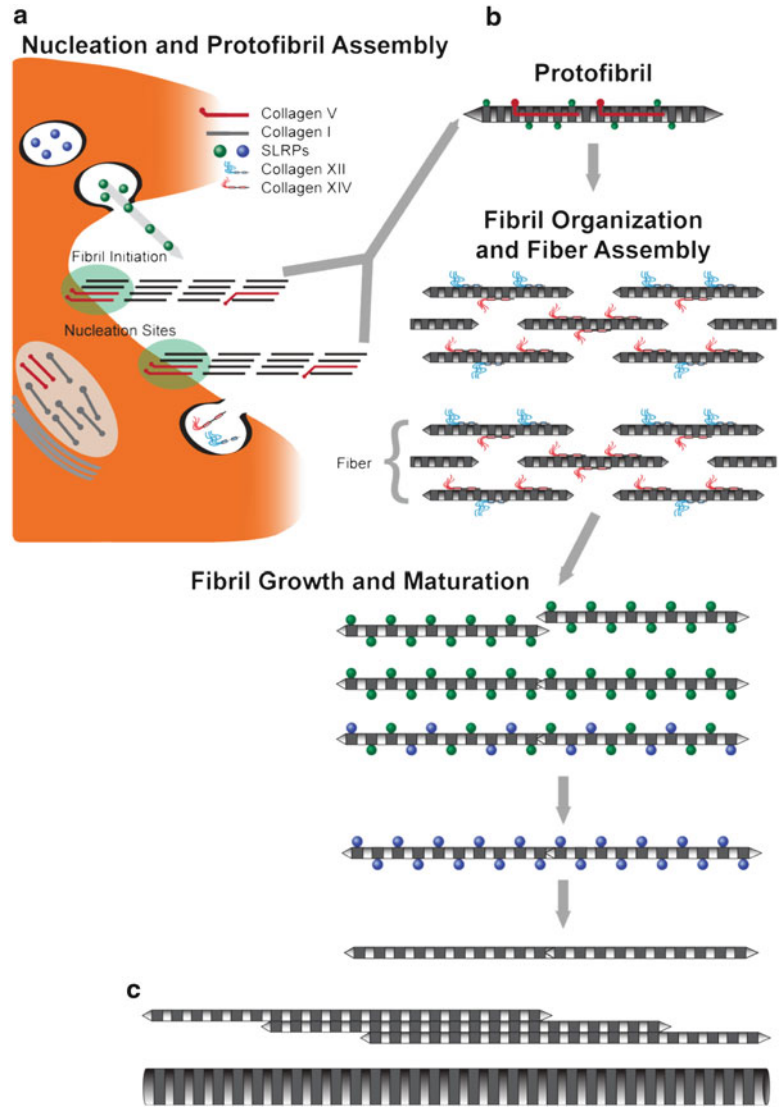

Fig. 2.6 Model of the regulation of fibril assembly. Model of the regulation of fibril assembly. Fibril assembly involves a sequence of events. (**a**) First, nucleators (e.g., collagen V and XI) initiate fibril assembly at the fibroblast cell surface. Then immature, small diameter, short protofibrils are assembled. The nucleation process is cell directed involving interactions with organizers (e.g., integrins and syndecans) at the cell surface. (**b**) Protofibrils are deposited into the matrix and are stabilized by interactions with regulators – other matrix components such as SLRPs and FACITs. (**c**) Changes in fibril stabilization resulting from processing, turnover and/or displacement regulate linear and lateral growth to mature fibrils in a tissue-specific manner (This figure has been adapted from Birk and Bruckner [131])

role for collagen V/XI in nucleation of assembly of short, small diameter protofibrils.

Under physiological conditions in vitro, collagens I and II can self-assemble after long lag phases. The nucleation of fibril formation by collagen V or XI provides a mechanism for the fibroblast to define the site of fibril formation. That is, the number of originating fibrils is controlled by the number of nucleation sites provided by the fibroblast. This feature is tissue-specific. For example, in the cornea where smaller fibril diameters are paramount to transparency,

collagen V is 10–20 % of the fibril-forming collagen content, and the many nucleation sites contribute to many smaller diameter fibrils [97, 98]. However, in tendons where mechanical strength depends upon larger diameter fibrils, collagen V makes up 1–5 % of the fibril-forming collagen content. One classic example of the collagen I/V interaction and its impact on structure is best seen in the classic form of Ehlers-Danlos Syndrome, a generalized connective tissue disorder where the majority of patients are heterozygous for mutations in collagen V [99]. This disorder results in approximately 50 % of the normal collagen V. Affected patients have a dermal phenotype with large, structurally aberrant fibrils. Similarly, in the heterozygous (*Col5a1+/−*) mouse model, there was a reduction of 50 % in collagen V fewer fibrils assembled, indicating fewer nucleation events than the normal mice [91], a similar situation occurs in the tendon [21]. The reduction in nucleation sites makes collagen V a rate limiting molecule in this instance. The regulation of these collagen I/V interactions is coordinated by the domain structure at the sites of assembly and by other molecules that organize and sequester these interactions at the cell surface [9].

The nucleation of protofibrils involves direction from cellular structures such as organelles, cytoskeletal components, cytoplasmic membrane domains, as well as organizing molecules. However, equally important are cell-defined extracellular domains. Without such direction, fibril assembly is inefficient. For example, in monolayer cultures, in the absence of extracellular matrix, procollagens are secreted freely into the media. Thus, organizing molecules provide a resource for tissue-specific coupling of fibril assembly to the cell surface. Consequently, cell-directed positioning of the deposited matrix is possible as assembled protofibrils undock from the cell surface, are extruded from the cell, and are incorporated into the extracellular matrix so that nucleation sites might be re-primed for the next round of nucleation.

Cell-directed collagen fibril assembly involves organizing molecules such as integrins and fibronectin. Fibronectin mediates the cell's interaction with assembling collagen fibrils as well as with other extracellular matrix molecules. Fibronectin molecules assemble into fibrils via integrin interactions [87, 100, 101]. The fibronectin fibril network contains multiple binding sites for collagen fibril assembly. When binding sites are blocked in fibronectin networks, collagen fibril assembly is inhibited [102]. Moreover, modifications to fibronectin-integrin interaction affect collagen fibril assembly, suggesting both that the cytoskeleton is involved in some way with collagen fibril assembly and that other direct interactions of integrins with other surface molecules are essential to fibril assembly.

Once the protofibrils are assembled and deposited into the extracellular matrix, further assembly to the mature fibrils involves linear and lateral growth of the preformed intermediates. In tendons and ligaments, numerous molecules are involved in the regulation of these steps. Two classes of regulatory molecules are the small leucine-rich proteoglycans (SLRPs) and the FACIT collagens. Both classes are fibril-associated and molecules within each class have their own tissue-specific, temporal, and spatial expression patterns. Differences in expression patterns contribute to the differences in structure and function amongst tissues, including tendons and ligaments.

Small leucine rich proteoglycans (SLRPs) are important regulators of linear and lateral fibril growth [103]. Two classes of SLRPs are expressed throughout tendon growth and maturation. They are divided into class I (decorin and biglycan) and class II (fibromodulin and lumican). When the genes for these molecules are specifically targeted via single or compound SLRP deficient mice, their importance in regulation of linear and lateral fibril growth in tendons is demonstrated [103–109]. In tendons, decorin and fibromodulin are dominant in this regulation, and they can be modulated by biglycan and lumican, respectively [88, 110]. A lack of decorin, biglycan, or fibromodulin leads to disruptions in fibril growth, resulting in larger diameter fibers, structural abnormalities, and biomechanical alterations in the tendon [88, 104, 111–114]. Moreover, synergistic (additive) effects are seen between classes when compound biglycan and

fibromodulin deficiencies occur that affect fibril diameters, alter tendon biomechanics, and even promote ectopic ossification within the tendon [104, 111, 115]. Unlike fibril-forming collagens, SLRPs regularly turnover and more easily allow for changes in expression to affect fibrillogenesis and tendon structure throughout development, maturation, and injury. A more detailed description of activities mediated by SLRPs can be found in Chap. 4.

The regulation of linear and lateral fibril growth is also affected by FACIT collagens. As described earlier in the chapter, FACIT collagens are fibril-associated molecules with large non-collagenous domains. Like SLRPs, FACITs also demonstrate differing tissue-specific and temporal expression patterns. Collagen IX is involved in regulation of fibril growth in cartilage [23]. Collagen XIV has recently been implicated in the regulation of tendon fibril growth [25]. Targeted deletion of collagen XIV in mouse models demonstrated a premature entrance into the fibril growth stage in tendons, resulting in larger diameter fibrils in early developmental stages. This indicates that in some tissues like tendons FACITs may serve as 'gate keepers' regulating the transition from protofibril assembly to fibril growth during development. In the case of collagen XIV, it temporarily stabilizes protofibrils to prevent the initiation of lateral fibril growth. The large non-collagenous domain and its inter-fibrillar location have been long suspected to play an additional role in fibril packing. Control of fibril packing also would influence lateral associations necessary for growth.

2.14 Effects of Composition and Structure on Function for Tendons and Ligaments

Tendons and ligaments are dense connective tissues composed of similar proteins and other macromolecules. Both tissues have a large extracellular matrix to cell ratio. However, slight differences in content and morphology allow for each tissue type to function in a distinct manner. The composition of the extracellular matrix in tendon is crucial for transmitting the force that is generated from muscle to bone. In contrast, the ligament has a more passive role of attaching bone to bone, guiding the joints motion, and preventing abnormal displacement of bones; to a minor extent, ligaments must also withstand load to resist joint instability. Collagens make up 60–70 % and 70–80 % of the dry weight for ligaments and tendons, respectively [1, 116]. The predominant fibril-forming collagen of tendons and ligaments is collagen I; other collagens within tendons and ligaments include III, V, VI, XII, and XIV. Besides collagens, tendons and ligaments contain many of the macromolecules mentioned throughout this chapter that regulate fibril assembly as well as water (60–70 % in ligaments and 50–60 % in tendons) [116]. Thus, while the content of these tissues is quite similar, there are distinctions in proportional compositions of water and macromolecules. Likewise, there are several similarities in the morphologies of the two tissues. Both tendons and ligaments have many parallel fibers that run along the axis of tension and these fibers are composed of mainly fibrils containing collagen I. There is also a "crimping" pattern in the collagen fibers of both tissues. However, there are several morphological distinctions. For example, within ligaments there are many regions where collagen fibers intertwine with adjacent fibers so that subsets of fibers are running obliquely at a 20–30° angle from the other fibers running along one axis [117]. These differences in composition and morphology are enough to contribute to the function of the tissues.

Composition of both tendons and ligaments contributes to the nonlinear anisotropic mechanical behavior that is exhibited in both tissues. An example load-elongation curve is depicted in Fig. 2.7. When loading conditions are low, tendons and ligaments are relatively compliant. In the "toe" region of the curve, initial load is affecting the "crimped" collagen fibers and any viscoelastic properties provided by molecules interacting with the collagens. The "toe" regions of the tendon is smaller because most collagen fibers are oriented parallel to the directions of strain and thus less realignment is necessary. With increasing

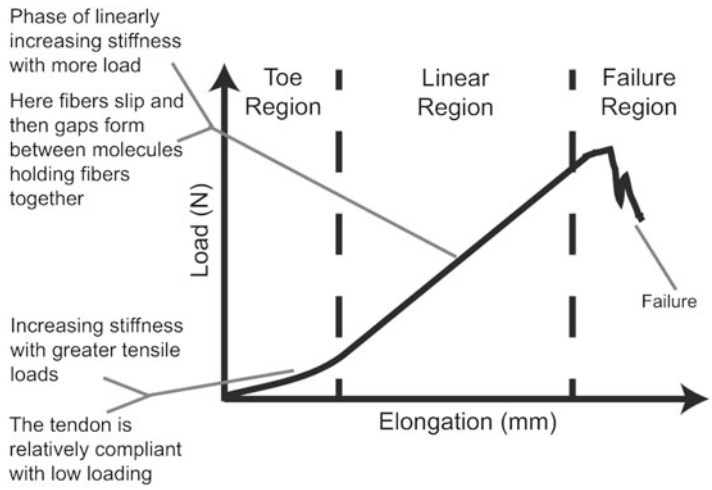

Fig. 2.7 Load-elongation curve for tendons and ligaments. There are three distinct regions within the curve which define the response to tensile loading. In the "Toe Region," initial load is affecting the "crimped" collagen fibers and any viscoelastic properties provided by molecules interacting with the collagens. In the "Linear Region," increasing tensile loads cause lengthening as the tendon or ligament becomes increasingly stiffer. In this region, load/elongation will ultimately follow a linear slope as slippage occurs within and then between collagen fibrils. In the "Failure Region," load continues to increase until the point of tearing as adjacent fibril molecules slip away to tensile failure, or complete rupture

tensile loads, these tissues – tendons more so than ligaments – become increasingly stiffer. At some point, load/length, or stiffness, will follow a linear slope as slippage occurs within collagen fibrils, next between fibrils, and then until the point of tearing as adjacent fibril molecules slip away with tensile failure (termed, ultimate load or point of ultimate tensile stress). As the load proceeds from initial strain to the point of failure, the area under the curve is considered the total energy absorbed.

Though both tendons and ligaments are considered to exhibit a nonlinear anisotropic mechanical behavior, differences in their load-elongation curves do exist. As already mentioned, the morphology of the ligament allows for the "toe" of the curve from the initial loading to be longer because the crimping pattern of the ligament is more greatly affected by fibrils not oriented exactly parallel – sometimes even perpendicular to the direction of the load. The stiffness of tendons and ligaments are also distinct. In humans, the elastic modulus of a tendon (Achilles tendon, 375 ± 102 MPa; biceps tendon, 421 ± 212 MPa; patellar tendon, 660 ± 266 MPa) is generally greater than that of a ligament (meniscofemoral ligament, 355 ± 234 MPa; anterolateral bundle of the posterior cruciate ligament, 294 ± 115 MPa; posterior bundle of the posterior cruciate ligament, 150 ± 69 MPa) [118, 119]. Moreover the stiffness for tendons (Achilles tendon 430 N/mm) is generally greater than for ligaments (lateral/medial collateral ligaments, 20 N/mm; anterior cruciate ligament, ACL 182 N/mm) [120–122]. Greater values for elastic modulus and stiffness are indicative of stiffer, less flexible connective tissue that is capable of absorbing and transmitting more energy. In addition, the tensile strength of tendons (50–150 N/m^2) is greater than that of ligaments (26–39 N/m^2) [123–125]. The compositional differences and distinctions in stiffness and tensile strength between tendons and ligaments all contribute to the understanding of how these two connective tissues function.

Tendons and ligaments have different roles. Tendons center the actions of several muscles into one axis of stress or strain. They distribute contractile force of muscles to bones, and they provide the muscle with distance from the insertion

that might mechanically beneficial. Tendons store elastic energy during locomotion, and prevent muscle injury with viscoelasticity. The relative stiffness and tensile strength of tendons is essential for maintaining force transmission. Ligaments, on the other hand, guide joint motion by attaching adjacent bones involved. This stabilizes the joint and controls the range of motion when load is applied. Their flexibility, relative to tendons, allows for range of motion within the joint. Tendons are susceptible to injury from overuse, wear and tear, and abrupt tears or avulsions when great forces are applied. Ligaments, though flexible, have less tensile strength and are prone to shear force injuries. The functions of tendons and ligaments are made manifest by thorough consideration of their composition, morphology, and physiology.

While tendons and ligaments possess characteristics that might distinguish one connective tissue from another, each of these tissue types also differs by anatomical location. For example, features of an Achilles tendon are not identical to those of a flexor digitorus profundus tendon or a patellar tendon. Likewise, characteristics of an anterior cruciate ligament are not identical to those of a medial collateral ligament. In addition, within each tendon and ligament, there are zones where composition changes. For example, ligaments can be divided into ligament mid-substance, fibrocartilage, mineralized fibrocartilage, and bone. Similarly, tendons have musculotendinous junctions, mid-substance, fibrocartilage, and mineralized fibrocartilage to the enthesis. In the following sections, differences in collagen structure and physiology will be described by anatomic location and by zone.

2.15 Effect of Anatomical Location on Tendons and Ligaments

While most tendons and ligaments are generally composed of the same content described throughout the chapter, there are slight differences in gross structure and content that might better allow the tendon to function at its anatomical location [1]. Tendons like that of the flexor digitorus profundus are round and contain the typical parallel bundles of collagen fibers. However, tendons such as those in the rotator cuff are flat, layered, and multi-directional; each tendon contains parallel collagen fibers as well as fibers that interdigitate obliquely with fibers from other tendons within the rotator cuff [126]. Rotator cuff tendons also contain more proteoglycans throughout than the typical round tendons; additional proteoglycans are believed to be aggrecan and SLRP biglycan [127]. A study of ovine tendons and ligaments demonstrated in an extracellular matrix analysis that each tendon (long digital extensor tendon, superficial digital flexor tendon, patellar tendon) and ligament (lateral collateral ligament, medial collateral ligament, posterior cruciate ligament, anterior cruciate ligament) had its own unique range for matrix compositions when examining water, glycosaminoglycan, and collagen content [116]. Moreover, each tendon and ligament demonstrates its own collagen organizational and mechanical features [116]. Gross anatomical differences in tendon and ligament size and shape occur as these connective tissues: traverse areas with limited space (e.g., within the wrist), centralize the force of several muscles (e.g., the Achilles tendon), or manage multi-directional forces and movements by intertwining collagen fibers with fibers of nearby connective tissues associated with tension in another axis (e.g., cruciate ligaments and rotator cuff tendons). The precise composition and structural arrangement of each tendon and ligament provides specific mechanical properties to allow that connective tissue to function. Thus, to some extent, each tendon and ligament has its own unique features.

2.16 Roles of Collagens in Transition from Midsubstance to Enthesis in Tendons and Ligaments

Much of what has been described in this chapter to this point has covered the tendon and ligament mid-substance. The transition of tendons

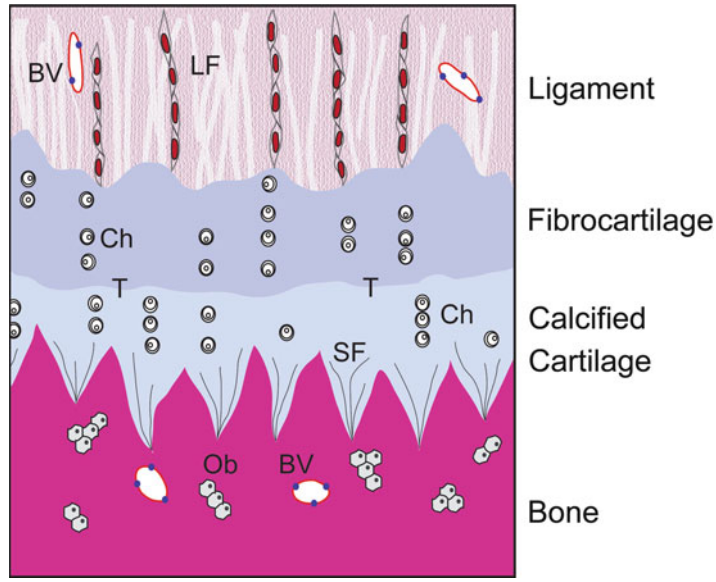

Fig. 2.8 Transition zones. This illustration demonstrates the transition from fibrous tissue to bone at a ligament insertion. The tissue transitions from ligament to fibrocartilage. Increases in the level of calcification are noted in the compositional gradient closer to insertion; this compositional change is demarcated by a tidemark (*T*) which traces the interface between non-calcified fibrocartilage and the next zone, calcified fibrocartilage. Calcified fibrocartilage interdigitates with the underlying subchondral bone to complete the insertion. Structures of note within the illustration include: ligament fibroblasts (LF), fibrocartilage chondrocytes (Ch), osteoblasts (Ob), Sharpey's fibers (SF), and blood vessels (BV) (This figure has been adapted from Place et al. [132])

and ligaments toward entheses are associated with changes in their composition and organization. There is a transition from tendinous and ligamentous material to bone (Fig. 2.8). These transition sites are not simple discreet units; instead, there is more of a gradient of molecular differences from mid-substance to bone. That said, one can identify general zones: mid-substance, fibrocartilage, calcified fibrocartilage, and bone [128]. The first zone consists of mid-substance or tendon proper; its composition has been described throughout the chapter. Basically, this zone contains collagen I-rich fibers that are aligned parallel to one another along the axis of strain, as well as a small amount of collagens V, VI, XII, and XIV, decorin, and other matrix macromolecules. The second zone is best characterized as fibrocartilage. It is predominantly composed of collagens II and III with minor amounts of collagens I, IX, and X, as well as aggrecan and decorin [129]. The composition of the second zone departs from that of the tendon or ligament-midsubstance. The third zone contains mineralized or calcified fibrocartilage. It is primarily composed of collagen II with significant amounts of collagen X and aggrecan [130]. The fourth zone is characterized as bone; it is predominantly composed of collagen I as well as components typically found within bone. The continuity of the enthesis is an efficient way to transfer and buffer loads applied between muscle, tendon, and bone or from bone to bone. The structure and composition of the region from mid-substance to fibrocartilage accommodates loads along the axis of the tendon, while the region with mineralized fibrocartilage and bone zones manages complex multidirectional forces that occur nearer to the bone [130]. The gradation of this transitional structure is difficult to replicate in surgery with native of engineered grafts.

2.17 Summary

The functions of tendons and ligaments depend greatly upon their extracellular matrices, particularly the collagen that comprises 70–80 % of their dry weight. Collagens found within tendons and ligaments belong to several subfamilies that can be grouped by their predominant suprastructural forms; these include: fibril-forming, FACIT, basement membrane, and beaded filament-forming collagens. The many collagens provide considerable diversity for the functional extracellular matrix suprastructures. This diversity is further compounded by the numbers of different alpha chains formed; by alternative splicing; as well as by the many types of post-translational modifications. Overall it is the suprastructural organization of these collagen molecules that provides tissue-specific structure and function, particularly to tendons and ligaments. These suprastructures are macromolecular heteropolymers containing different collagens and other fibril-associated molecules. Within tendons and ligaments, the assembly of these collagen suprastructures relies upon the primary structures of these molecules that contain domains that will affect downstream secondary, tertiary, and quaternary structures. Clearly the content and organization of these connective tissues affect how they function in absorbing and transmitting forces as well in maintaining stability. While generalizations can be made for both connective tissues, current studies demonstrate that differences exist between different tendons as well as between tendons and ligaments. Each tissue's basic composition and structure will affect its unique physiology. Thus, while an understanding of how collagen is assembled and organized is critical for tendon and ligament repair and regeneration, it is also essential to focus on how these general mechanisms generate unique structures that will determine each tissue's distinct features and functional properties.

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