Collagens—structure, function, and biosynthesis

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Abstract

The extracellular matrix represents a complex alloy of variable members of diverse protein families defining structural integrity and various physiological functions. The most abundant family is the collagens with more than 20 different collagen types identified so far. Collagens are centrally involved in the formation of fibrillar and microfibrillar networks of the extracellular matrix, basement membranes as well as other structures of the extracellular matrix. This review focuses on the distribution and function of various collagen types in different tissues. It introduces their basic structural subunits and points out major steps in the biosynthesis and supramolecular processing of fibrillar collagens as prototypical members of this protein family. A final outlook indicates the importance of different collagen types not only for the understanding of collagen-related diseases, but also as a basis for the therapeutical use of members of this protein family discussed in other chapters of this issue.
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Contents

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1. Collagens—general introduction

The extracellular matrix of connective tissues represents a complex alloy of variable members of diverse protein families defining structural integrity and various physiological functions. The supramolecular arrangement of fibrillar elements, microfibrillar networks as well as soluble proteins, glycoproteins and a wide range of other molecules define the biophysical characteristics. Composition and structure vary considerably among different types of connective tissues. Tissue-specific expression and synthesis of structural proteins and glycoprotein components result in the unique functional and biological characteristics at distinct locations.

The primary function of extracellular matrix is to endow tissues with their specific mechanical and biochemical properties. Resident cells are responsible for its synthesis and maintenance, but the extracellular matrix, in turn, has also an impact on cellular functions. Cell–matrix interactions mediated by specific cell receptors and cell binding epitopes on many matrix molecules do not only play a dominant role in cell attachment and migration, but also regulate or promote cellular differentiation and gene expression levels. The pericellular matrix provides a special physiological microenvironment for the cells protecting them from detrimental mechanical influences and also mediating mechanically induced signal transmission. An additional influence of the extracellular matrix on morphogenesis and cellular metabolism can be ascribed to the storage and release of growth factors which is modulated by their binding to specific matrix components [1,2].

The most abundant proteins in the extracellular matrix are members of the collagen family. Collagens were once considered to be a group of proteins with a characteristic molecular structure with their fibrillar structures contributing to the extracellular scaffolding. Thus, collagens are the major structural element of all connective tissues and are also found in the interstitial tissue of virtually all parenchymal organs, where they contribute to the stability of tissues and organs and maintain their structural integrity. However, in the last decade, the knowledge increased and the collagen family expanded dramatically (Table 1). All members are characterized by containing domains with repetitions of the proline-rich tripeptide Gly-X-Y involved in the formation of trimeric collagen triplehelices. The functions of this heterogeneous family are not confined to provide structural components of the fibrillar backbone of the extracellular matrix, but a great variety of additional functional roles are defined by additional protein domains.

The knowledge about the molecular structure, biosynthesis, assembly and turnover of collagens is important to understand embryonic and fetal developmental processes as well as pathological processes linked with many human diseases. The exploration of expression and function of the different collagen types also contributes to a better understanding of diseases which are based on molecular defects of collagen genes such as chondrodysplasias, osteogenesis imperfecta, Alport syndrome, Ehler's Danlos Syndrome, or epidermolysis bullosa [3,4]. Additionally, collagen degradation and disturbed metabolism are important in the course of osteoarthritis and osteoporosis. A profound knowledge of the properties of the different types of collagens may also be beneficial in therapeutical aspects. Due to their binding capacity, they could serve as delivery systems for drugs, growth factors or cells and the network-forming capacity and anchoring function of certain collagen types could contribute to the formation of scaffolds promoting tissue repair or regeneration [2,5,6].

2. Collagens—the basic structural module

The name ''collagen'' is used as a generic term for proteins forming a characteristic triple helix of three polypeptide chains and all members of the collagen family form these supramolecular structures in the

Table 1
Table showing the various collagen types as they belong to the major collagen families

Type	Molecular composition	Genes (genomic localization)	Tissue distribution
Fibril-forming collagens			
I	$[\alpha 1(I)]_2\alpha 2(I)$	COL1A1 (17q21.31–q22)	bone, dermis, tendon, ligaments, cornea
		COL1A2 (7q22.1)	
II	$[\alpha 1(II)]_3$	COL2A1 (12q13.11–q13.2)	cartilage, vitreous body, nucleus pulposus
III	$[\alpha 1(III)]_3$	COL3A1 (2q31)	skin, vessel wall, reticular fibres of most tissues (lungs, liver, spleen, etc.)
V	$\alpha 1(V),\alpha 2(V),\alpha 3(V)$	COL5A1 (9q34.2–q34.3)	lung, cornea, bone, fetal membranes; together with type I collagen
		COL5A2 (2q31)	
		COL5A3 (19p13.2)	
XI	$\alpha 1(XI)\alpha 2(XI)\alpha 3(XI)$	COL11A1 (1p21)	cartilage, vitreous body
		COL11A2 (6p21.3)	
		COL11A3 = COL2A1	
Basement membrane collagens			
IV	$[\alpha 1(IV)]_2\alpha 2(IV); \alpha 1-\alpha 6$	COL4A1 (13q34)	basement membranes
		COL4A2 (13q34)	
		COL4A3 (2q36–q37)	
		COL4A4 (2q36–q37)	
		COL4A5 (Xq22.3)	
		COL4A6 (Xp22.3)	
Microfibrillar collagen			
VI	$\alpha 1(VI),\alpha 2(VI),\alpha 3(VI)$	COL6A1 (21q22.3)	widespread: dermis, cartilage, placenta, lungs, vessel wall,
		COL6A2 (21q22.3)	intervertebral disc
		COL6A3 (2q37)	
Anchoring fibrils			
VII	$[\alpha 1(VII)]_3$	COL7A1 (3p21.3)	skin, dermal–epidermal junctions; oral mucosa, cervix,
Hexagonal network-forming collagens			
VIII	$[\alpha 1(VIII)]_2\alpha 2(VIII)$	COL8A1 (3q12–q13.1)	endothelial cells, Descemet's membrane
		COL8A2 (1p34.3–p32.3)	
X	$[\alpha 3(X)]_3$	COL10A1 (6q21–q22.3)	hypertrophic cartilage
FACIT collagens			
IX	$\alpha 1(IX)\alpha 2(IX)\alpha 3(IX)$	COL9A1 (6q13)	cartilage, vitreous humor, cornea
		COL9A2 (1p33–p32.2)	
XII	$[\alpha 1(XII)]_3$	COL12A1 (6q12–q13)	perichondrium, ligaments, tendon
XIV	$[\alpha 1(XIV)]_3$	COL9A1 (8q23)	dermis, tendon, vessel wall, placenta, lungs, liver
XIX	$[\alpha 1(XIX)]_3$	COL19A1 (6q12–q14)	human rhabdomyosarcoma
XX	$[\alpha 1(XX)]_3$		corneal epithelium, embryonic skin, sternal cartilage, tendon
XXI	$[\alpha 1(XXI)]_3$	COL21A1 (6p12.3–11.2)	blood vessel wall
Transmembrane collagens			
XIII	$[\alpha 1(XIII)]_3$	COL13A1 (10q22)	epidermis, hair follicle, endomysium, intestine, chondrocytes, lungs, liver
XVII	$[\alpha 1(XVII)]_3$	COL17A1 (10q24.3)	dermal–epidermal junctions
Multiplexins			
XV	$[\alpha 1(XV)]_3$	COL15A1 (9q21–q22)	fibroblasts, smooth muscle cells, kidney, pancreas,
XVI	$[\alpha 1(XVI)]_3$	COL16A1 (1p34)	fibroblasts, amnion, keratinocytes
XVIII	$[\alpha 1(XVIII)]_3$	COL18A1 (21q22.3)	lungs, liver

Given are the molecular composition, the genomic localization of the different chains as well as the basic tissue distribution.

extracellular matrix although their size, function and tissue distribution vary considerably. So far, 26 genetically distinct collagen types have been described [4,7–11].

Based on their structure and supramolecular organization, they can be grouped into fibril-forming collagens, fibril-associated collagens (FACIT), network-forming collagens, anchoring fibrils, transmembrane collagens, basement membrane collagens and others with unique functions (see Table 1).

The different collagen types are characterized by considerable complexity and diversity in their structure, their splice variants, the presence of additional, non-helical domains, their assembly and their function. The most abundant and widespread family of collagens with about 90% of the total collagen is represented by the fibril-forming collagens. Types I and V collagen fibrils contribute to the structural backbone of bone and types II and XI collagens predominantly contribute to the fibrillar matrix of articular cartilage. Their torsional stability and tensile strength lead to the stability and integrity of these tissues [4,12,13]. Type IV collagens with a more flexible triple helix assemble into meshworks restricted to basement membranes. The microfibrillar type VI collagen is highly disulfide cross-linked and contributes to a network of beaded filaments interwoven with other collagen fibrils [14]. *F*ibril-*a*ssociated *c*ollagens with *i*nterrupted *t*riplehelices (FACIT) such as types IX, XII, and XIV collagens associate as single molecules with large collagen fibrils and presumably play

a role in regulating the diameter of collagen fibrils [9]. Types VIII and X collagens form hexagonal networks while others (XIII and XVII) even span cell membranes [15].

Despite the rather high structural diversity among the different collagen types, all members of the collagen family have one characteristic feature: a right-handed triple helix composed of three α-chains (Fig. 1) [7,16]. These might be formed by three identical chains (homotrimers) as in collagens II, III, VII, VIII, X, and others or by two or more different chains (heterotrimers) as in collagen types I, IV, V, VI, IX, and XI. Each of the three α-chains within the molecule forms an extended left-handed helix with a pitch of 18 amino acids per turn [17]. The three chains, staggered by one residue relative to each other, are supercoiled around a central axis in a right-handed manner to form the triple helix [18]. A structural prerequisite for the assembly into a triple helix is a glycine residue, the smallest amino acid, in every third position of the polypeptide chains resulting in a (Gly-X-Y)$_n$ repeat structure which characterizes the ''collagenous'' domains of all collagens. The α-chains assemble around a central axis in a way that all glycine residues are positioned in the center of the triple helix, while the more bulky side chains of the other amino acids occupy the outer positions. This allows a close packaging along the central axis of the molecule. The X and Y position is often occupied by proline and hydroxyproline. Depending on the collagen type, specific proline and lysine residues are
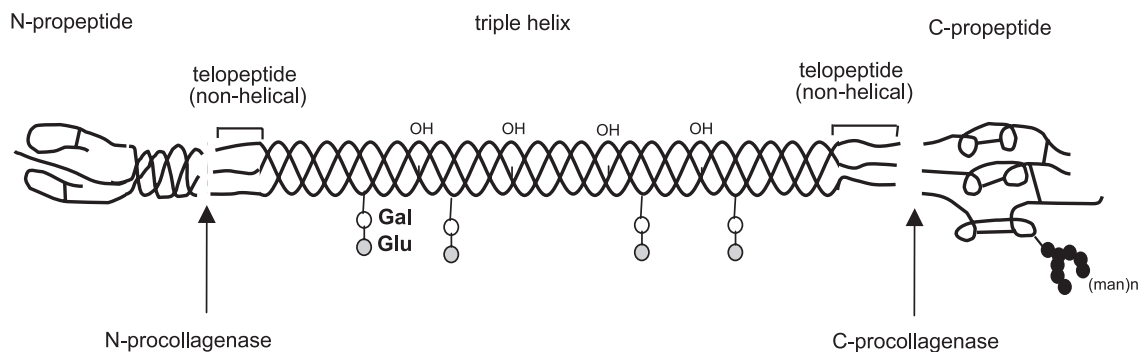

Fig. 1. Molecular structure of fibrillar collagens with the various subdomains as well as the cleavage sites for N- and C-procollagenases (shown is the type I collagen molecule). Whereas they are arranged in tendon in a parallel manner they show a rather network-like supramolecular arrangement in articular cartilage.

modified by post-translational enzymatic hydroxylation. The content of 4-hydroxyproline is essential for the formation of intramolecular hydrogen bonds and contributes to the stability of the triple helical conformation. Some of the hydroxylysines are further modified by glycosylation. The length of the triple helical part varies considerably between different collagen types. The helix-forming (Gly-X-Y) repeat is the predominating motif in fibril-forming collagens (I, II, III) resulting in triple helical domains of 300 nm in length which corresponds to about 1000 amino acids [3,4]. In other collagen types, these collagenous domains are much shorter or contain non-triple helical interruptions. Thus, collagen VI or X contains triple helices with about 200 or 460 amino acids, respectively [4]. Although the triple helix is a key feature of all collagens and represents the major part in fibril-forming collagens, non-collagenous domains flanking the central helical part are also important structural components (Fig. 1). Thus, the C-propeptide is thought to play a fundamental role in the initiation of triple helix formation, whereas the N-propeptide is thought to be involved in the regulation of primary fibril diameters [3]. The short non-helical telopeptides of the processed collagen monomers (see Fig. 1) are involved in the covalent cross-linking of the collagen molecules as well as linking to other molecular structures of the surrounding matrix [38].

FACIT collagens are characterized by several non-collagenous domains interrupting the triple helices, which may function as hinge regions [19]. In other collagens like collagens IV, VI, VII, VIII or X, non-collagenous domains are involved in network formation and aggregation. In contrast to the highly conserved structure of the triple helix, non-collagenous domains are characterized by a more structural and functional diversity among different collagen families and types. Interruptions of the triple helical structure may cause intramolecular flexibility and allow specific proteolytic cleavage. Native triple helices are characterized by their resistance to proteases such as pepsin, trypsin or chymotrypsin [20] and can only be degraded by different types of specific collagenases. Collagenase A (MMP-1) [21], the interstitial collagenase, is expressed by a large variety of cells and is thought to be centrally involved in tissue remodeling, e.g. during wound healing. MMP-8 (collagenase B) is largely specific for neutrophil granulocytes [22] and, thus, thought to be mainly involved in tissue destruction during acute inflammatory processes. MMP-13 (collagenase C) [23] is expressed by hypertrophic chondrocytes as well as osteoblasts and osteoclasts [24] and therefore most likely plays an important role in cartilage and bone remodeling. Many other matrix metalloproteinases are able to cleave the denatured collagen ("gelatin"). The detailed analysis of the interplay of MMPs as well as specific inhibitors will describe the reactivities in vivo as well as potential pharmaceutical options for intervention [25–27].

3. Distribution, structure, and function of different collagen types

3.1. Collagen types I, II, III, V and XI—the fibril-forming collagens

The classical fibril-forming collagens include collagen types I, II, III, V, and XI. These collagens are characterized by their ability to assemble into highly oriented supramolecular aggregates with a characteristic suprastructure, the typical quarter-staggered fibril-array with diameters between 25 and 400 nm (Fig. 2). In the electron microscope, the fibrils are defined by a characteristic banding pattern with a periodicity of about 70 nm (called the D-period) based on a staggered arrangement of individual collagen monomers [28].

Type I collagen is the most abundant and best studied collagen. It forms more than 90% of the organic mass of bone and is the major collagen of tendons, skin, ligaments, cornea, and many interstitial connective tissues with the exception of very few tissues such as hyaline cartilage, brain, and vitreous body. The collagen type I triple helix is usually formed as a heterotrimer by two identical α1(I)-chains and one α2(I)-chain. The triple helical fibres are, in vivo, mostly incorporated into composite containing either type III collagen (in skin and reticular fibres) [29] or type V collagen (in bone, tendon, cornea) [30]. In most organs and notably in tendons and fascia, type I collagen provides tensile stiffness and in bone, it defines considerable biomechanical properties concerning load bearing, tensile
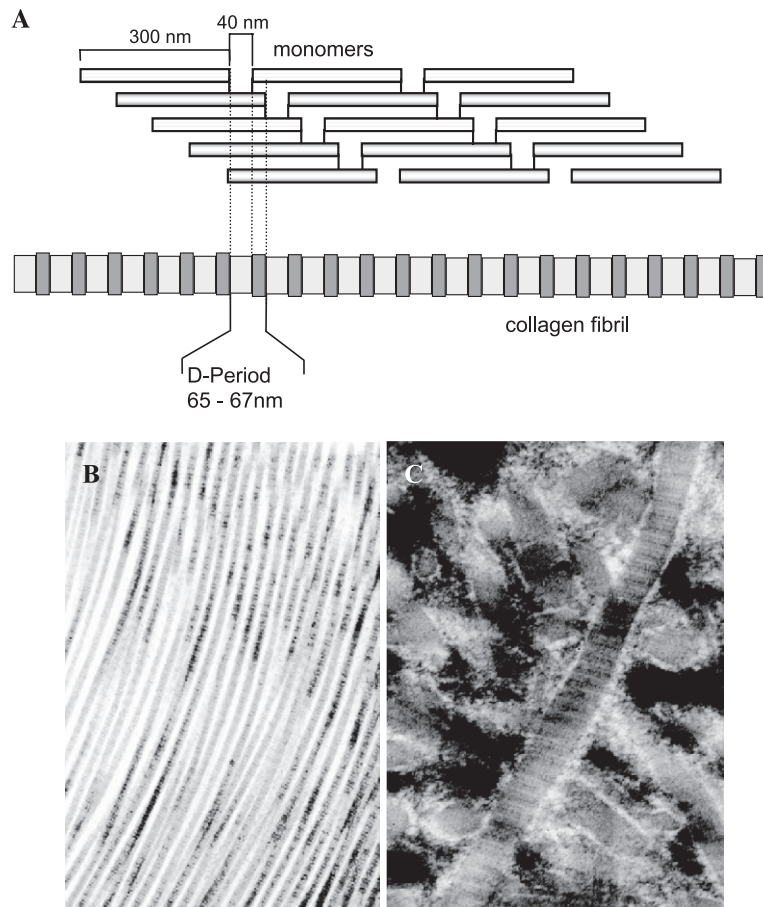
Fig. 2. (A) Schematic representation of the supramolecular assembly of the collagen fibrils in the characteristic quarter-staggered form. The monomers are 300-nm long and 40-nm gaps separate consecutive monomers causing the characteristic appearance of the collagen type I fibrils on the ultrastructural level. (B + C) Collagen type I (B) and II (C) fibrils as they are arranged in normal tendon (B) and articular cartilage (C). Whereas they are arranged in tendon in a parallel manner, they show a rather network-like supramolecular arrangement in articular cartilage.

strength, and torsional stiffness in particular after calcification.

The fibril-forming type II collagen is the characteristic and predominant component of hyaline cartilage. It is, however, not specifically restricted to cartilage where it accounts for about 80% of the total collagen content since it is also found in the vitreous body, the corneal epithelium, the notochord, the nucleus pulposus of intervertebral discs, and embryonic epithelial–mesenchymal transitions [4]. The triple helix of type II collagen is composed of three $\alpha1(II)$-chains forming a homotrimeric molecule similar in size and biomechanical properties to that of type I collagen [31]. Collagen fibrils in cartilage represent heterofibrils containing in addition to the dominant collagen II, also types XI and IX collagens which are supposed to limit the fibril diameter to about 15–50 nm [32] as well as other non-collagenous proteins. Compared to type I collagen, type II collagen chains show a higher content of hydroxylysine as well as glucosyl and galactosyl residues which mediate the interaction with proteoglycans, another typical component of the highly hydrated matrix of hyaline cartilage [13]. Alternative splicing of the type II collagen pre-mRNA results in two forms of the $\alpha1(II)$-chains. In the splice variant IIB,

the dominant form in mature cartilage, the second exon coding for a globular cystein-rich domain in the N-terminal propeptide is excluded, whereas it is retained in the IIA variant, the embryonic form found in prechondrogenic mesenchyme [33,34], osteophytes [35,36], perichondrium, vertebrae [33] and chondrogenic tumors [37]. The switch from IIA to IIB suggests a role during developmental processes and the IIB variant represents a characteristic marker for mature cartilage [3].

Type III collagen is a homotrimer of three α1(III)-chains and is widely distributed in collagen I containing tissues with the exception of bone [38]. It is an important component of reticular fibres in the interstitial tissue of the lungs, liver, dermis, spleen, and vessels. This homotrimeric molecule also often contributes to mixed fibrils with type I collagen and is also abundant in elastic tissues [39].

Types V and XI collagens are formed as heterotrimers of three different α-chains (α1, α2, α3). It is remarkable that the α3-chain of type XI collagen is encoded by the same gene as the α1-chain of type II collagen and only the extent of glycosylation and hydroxylation differs from α1(II) [4]. Although it is finally not sorted out, a combination between different types V and XI chains appears to exist in various tissues [40–43]. Thus, types V and XI collagens form a subfamily within fibril-forming collagens, though they share similar biochemical properties and functions with other members of this family. As mentioned before, type V collagen typically forms heterofibrils with types I and III collagens and contributes to the organic bone matrix, corneal stroma and the interstitial matrix of muscles, liver, lungs, and placenta [12]. Type XI collagen codistributes largely in articular cartilage with type II collagen [4,13]. The large amino-terminal non-collagenous domains of types V and XI collagens are processed only partially after secretion and their incorporation into the heterofibrils is thought to control their assembly, growth, and diameter [44]. Since their triple helical domains are immunologically masked in tissues, they are thought to be located central in the fibrils rather than on their surface [12,45]. Thus, type V collagen may function as a core structure of the fibrils with types I and III collagens polymerizing around this central axis. Analogous to this model, type XI collagen is supposed to form the core of

collagen II heterofibrils [3]. A high content of tyrosine-sulfate in the N-terminal domains of α1(V)- and α2(V)-chains, with 40% of the residues being O-sulfated, supports a strong interaction with the more basic triple helical part and is likely to stabilize the fibrillar complex [46].

3.2. Collagen types IX, XII, and XIV—The FACIT collagens

The collagen types IX, XII, XIV, XVI, XIX, and XX belong to the so-called *F*ibril-*A*ssociated *C*ollagens with *I*nterrupted *T*riple helices (FACIT collagens). The structures of these collagens are characterized by "collagenous domains" interrupted by short non-helical domains and the trimeric molecules are associated with the surfaces of various fibrils.

Collagen type IX codistributes with type II collagen in cartilage and the vitreous body [4]. The heterotrimeric molecule consists of three different α-chains (α1(IX), α2(IX), and α3(IX)) forming three triple helical segments flanked by four globular domains (NC1–NC4) [47]. Type IX collagen molecules are located periodically along the surface of type II collagen fibrils in antiparallel direction [48]. This interaction is stabilized by covalent lysine-derived cross-links to the N-telopeptide of type II collagen. A hinge region in the NC3 domain provides flexibility in the molecule and allows the large and highly cationic globular N-terminal domain to reach out from the fibril where it presumably interacts with proteoglycans or other matrix components [13,49]. A chondroitin-sulfate side chain is covalently linked to a serine residue of the α2(IX)-chain in the NC3 domain and the size may vary between tissues [50]. It might be involved in the linkage of various collagen fibres as well as their interaction with molecules of the extracellular matrix. Additionally, collagen type XVI is found in hyaline cartilage and skin [51] and is associated with a subset of the collagen "type II fibers" (Graessel, personal communication).

Types XII and type XIV collagens are similar in structure and share sequence homologies to type IX collagen. Both molecules associate or colocalize with type I collagen in skin, perichondrium, periosteum, tendons, lung, liver, placenta, and vessel walls [4]. The function of these collagens, as well as of collagen

types XIX [52] and XX [53], within the tissue is still poorly understood.

3.3. Collagen type VI—a microfibrillar collagen

Type VI collagen is an heterotrimer of three different α-chains (α1, α2, α3) with short triple helical domains and rather extended globular termini [54,55]. This is in particular true for the α3-chain which is nearly as twice as long as the other chains due to a large N- and C-terminal globular domains. However, these extended domains are subject not only to alternative splicing, but also to extensive posttranslational processing, both within and outside the cell [56,57]. The primary fibrils assemble already inside the cell to antiparallel, overlapping dimers, which then align in a parallel manner to form tetramers. Following secretion into the extracellular matrix, type VI collagen tetramers aggregate to filaments and form an independent microfibrillar network in virtually all connective tissues, except bone [14,57,58]. Type VI collagen fibrils appear on the ultrastructural level as fine filaments, microfibrils or segments with faint crossbanding of 110-nm periodicity [58–63], although not all fine filaments represent type VI collagen [64–68].

3.4. Collagen types X and VIII—short chain collagens

Types X and VIII collagens are structurally related short-chain collagens. Type X collagen is a characteristic component of hypertrophic cartilage in the fetal and juvenile growth plate, in ribs and vertebrae [7]. It is a homotrimeric collagen with a large C-terminal and a short N-terminal domain and experiments in vitro are indicative for its assembly to hexagonal networks [69]. The function of type X collagen is not completely resolved. A role in endochondral ossification and matrix calcification is discussed. Thus, type X collagen is thought to be involved in the calcification process in the lower hypertrophic zone [69–72], a possibility supported by 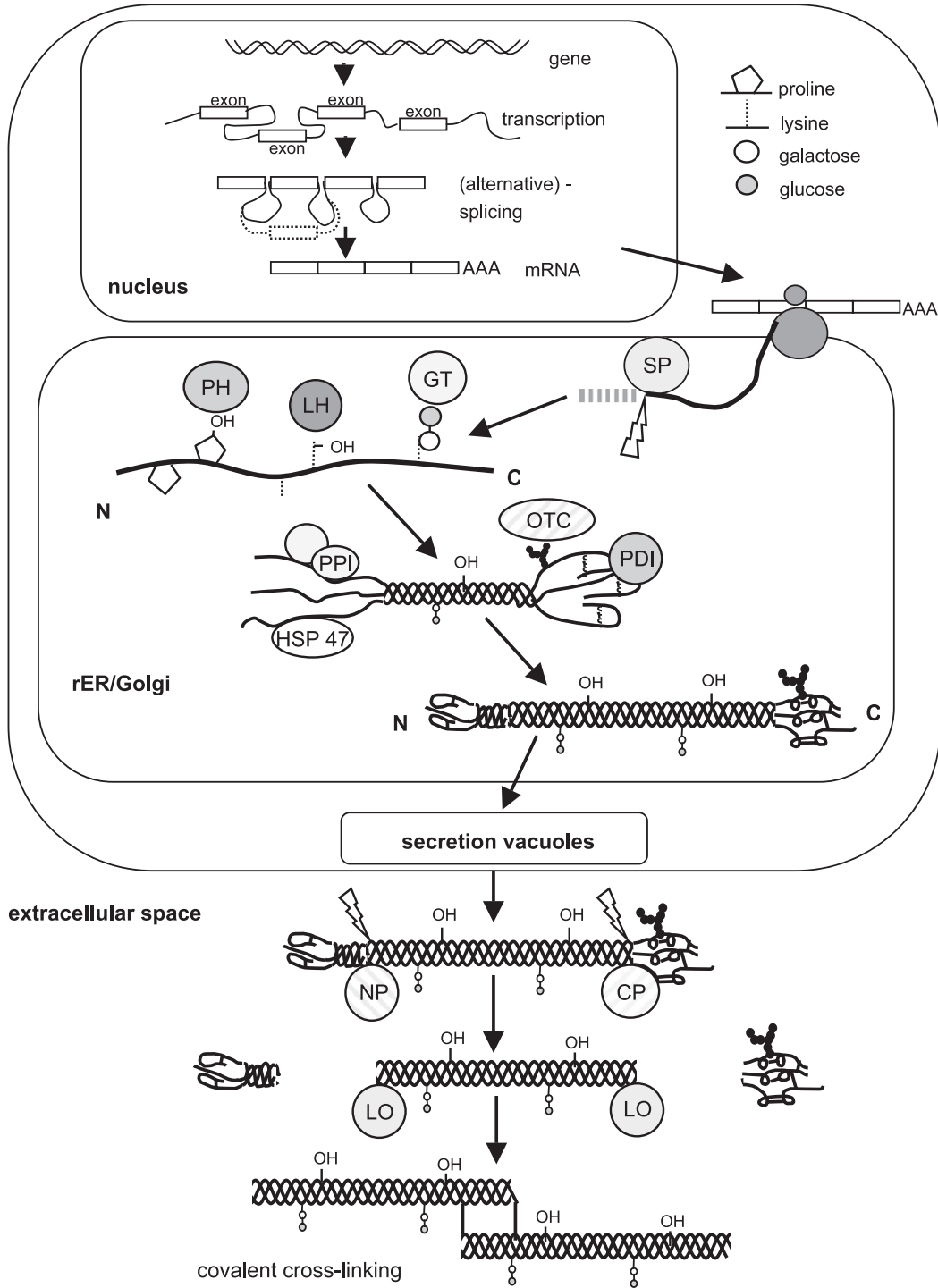the restricted expression of type X collagen in the calcified zone of adult articular cartilage [73,74] and its prevalence in the calcified chick egg shell [75]. In fetal cartilage, type X collagen has been localized in fine filaments as well as associated with type II fibrils. [76]. Mutations of the COL10A1 gene are causative for the disease Schmid type metaphyseal chondrodysplasia (SMCD) impeding endochondral ossification in the metaphyseal growth plate. This leads to growth deficiency and skeletal deformities with short limbs [77].

Type VIII collagen is very homologous to type X collagen in structure but shows a distinct distribution and may therefore have different functions [78]. This network-forming collagen is produced by endothelial cells and assembles in hexagonal lattices, e.g. in the Descemet's membrane in the cornea [79].

3.5. Collagen type IV—the collagen of basement membranes

Type IV collagen is the most important structural component of basement membranes integrating laminins, nidogens and other components into the visible two-dimensional stable supramolecular aggregate. The structure of type IV collagen is characterized by three domains: the N-terminal 7S domain, a C-terminal globular domain (NC1), and the central triple helical part with short interruptions of the Gly-X-Y repeats resulting in a flexible triple helix. Six subunit chains have been identified yet, α1(IV)−α6(IV), associating into three distinct heterotrimeric molecules. The predominant form is represented by α1(IV)$_2$α2(IV) heterotrimers forming the essential network in most embryonic and adult basement membranes. Specific dimeric interactions of the C-terminal NC1 domains, cross-linking of four 7S domains as well as interactions of the triple helical domains, are fundamental for the stable network of collagen IV [80]. The isoforms α3(IV)−α6(IV) show restricted, tissue-specific expression patterns and are forming either an independent homotypic network of α3(IV)α4(IV)α6(IV)

heterotrimers (kidney, lung) or a composite network of $\alpha5(IV)_2\alpha6(IV)/\alpha1(IV)_2\alpha2(IV)$ molecules [81]. Mutations of the major isoform $\alpha1(IV)_2\alpha2(IV)$ are assumed to be embryonic lethal, but defects of the $\alpha5(IV)$, as well as $\alpha3(IV)$ or $\alpha4(IV)$-chains are causative for various forms of Alport syndrome due to the importance of the $\alpha3\alpha4\alpha6$ heterotrimer for stability and function of glomerular and alveolar basement membranes [3].

4. Biosynthesis of collagens

The biosynthesis of collagens starting with gene transcription of the genes within the nucleus to the aggregation of collagen heterotrimers into large fibrils is a complex multistep process (Fig. 3). Since most of our knowledge of these mechanisms is based on fibril-forming collagens, this discussion will mostly focus on type I collagen. It is likely that the basic mechanisms of triple helix formation and processing will also apply for other collagen types.

4.1. Transcription and translation

The regulation of the transcriptional activities of collagens depends largely on the cell type, but may also be controlled by numerous growth factors and cytokines (for review, see Ref. [38]). Thus, bone formation is stimulated, at least in the adult, by members of the TGF-β-superfamily as well as the insulin-like-growth factors. In other tissues, fibroblast-growth-factors and many other agents are even more important. To discuss this in more detail is beyond the scope of this review and needs to be evaluated for all collagens and tissues separately.

Most collagen genes revealed a complex exon–intron pattern, ranging from 3 to 117 exons, with the mRNAs of fibrillar collagens encoded by more than 50 exons. Therefore, in many cases, different mRNA species could be detected, caused by multiple transcription initiation sites, alternative splicing of exons or combination of both. For example, in the cornea and the vitreous body, a shorter form of type IX collagen mRNA is generated by an additional start site between exons 6 and 7 [4]. Alternative splicing has been reported for many collagen types and was first described for type II collagen. A longer form of type II collagen (COL2A) is expressed by chondro-progenitor cells and varies from a shorter form (COL2B) where exon 2 is excluded [33] and which is the main gene product of mature articular chondrocytes. More recently, more than 17 different transcripts have been reported for type XIII collagen [82] and alternative splicing also generates heterogeneous transcription products for collagens VI, XI, XII [82–85]. In addition to splicing, the pre-mRNA undergoes capping at the 5′ end and polyadenylation at the 3′ end and the mature mRNA is transported to the cytoplasm and translated at the rough endoplasmatic reticulum.

Ribosome-bound mRNA is translated into preprocollagen molecules which protrude into the lumen of the rough endoplasmatic reticulum with the help of a signal recognition domain recognized by the corresponding receptors.

4.2. Posttranslational modifications of collagen

After removal of the signal peptide by a signal peptidase (Fig. 3), the procollagen molecules undergo multiple steps of post-translational modifications. Hydroxylation of proline and lysine residues catalyzed by prolyl 3-hydroxylase, prolyl 4-hydroxylase, and lysyl hydroxylase, respectively. All three enzymes require ferrous ions, 2-oxoglutarate, molecular oxygen, and ascorbate as cofactors. In fibril-forming collagens, approximately 50% of the proline residues contain a hydroxylgroup at position 4 and the extent of prolyl-hydroxylation is species-dependent. The organisms living at lower environmental temperatures show a lower extent of hydroxylation [86]. The presence of 4-hydroxyproline is essential for intramolecular hydrogen bonds and thus contributes to the thermal stability of the triple helical domain, and therefore also to the integrity of the monomer and collagen fibril. The function of 3-hydroxyproline is not known [3]. The extent of lysine hydroxylation also varies between tissues and collagen types [87]. Hydroxylysine residues are able to form stable intermolecular cross-linking of collagen molecules in fibrils and additionally represent sites for the attachment of carbohydrates. Glucosyl- and galactosyl-residues are transferred to the hydroxyl groups of hydroxylysine; this is catalyzed by the enzymes hydroxylysyl galactosyltransferase and galactosylhydroxylysyl-glucosyltransferase, respectively (Fig. 3).

The C-propeptides have an essential function in the assembly of the three α-chains into trimeric collagen monomers. The globular structure of the C-propeptides is stabilized by intrachain disulphide bonds and a N-linked carbohydrate group is added by the oligosaccharyl transferase complex. The formation to triple helices is preceded by the alignment of the C-terminal domains of three α-chains and initiates the formation of the triple helix progressing to the N-terminus. The efficient formation and folding of the procollagen chains depends on the presence of further enzymes like PPI (peptidyl-prolyl *cis-trans*-isomerase) [88] and collagen-specific chaperones like HSP47 [89]. The importance of these activities was substantiated by the pharmacological influence of cyclosporine A, an inhibitor of PPI-activity on the triple-helix formation in vitro [90,91] as well as the fatal consequences seen with a knock-out model of murine HSP47 [92]. Additionally, the enzyme protein disulphide isomerase PDI, identical with the β-subunit of prolyl 4-hydroxylase [93,94], is involved in the formation of intra- and interchain disulphide bonds in procollagen molecules [3].

4.3. Secretion of collagens

After processing and procollagen assembly, the triple-helical molecules are packaged within the Golgi compartment into secretory vesicles and released into the extracellular space. Following the secretion, the procollagen trimers are processed depending on the collagen type. The C-propeptides and N-propeptides are cleaved off by two specific proteases, the procollagen *N*-proteinase and the procollagen C-proteinase. Both proteins belong to a family of Zn^{2+}-dependent metalloproteinases [95] and the binding to the cell membrane and internalization of the released N- and C-propeptides was seen in studies of collagen-synthesizing fibroblasts [96]. Therefore, a feedback mechanism for the control of expression was discussed [3], suggesting a collagen-type specific modulating effect of the propeptides on the collagen synthesis by inhibiting chain initiation [97]. However, due to the lack of further studies, the mechanism and their physiological relevance remain unclear. Another study showed that the C-propeptide of type I collagen is internalized by fibroblasts and becomes localized within the nucleus [98]. A potential effect on transcription was discussed, but again, the potential

mechanisms of regulation remained largely unresolved [3].

4.4. Extracellular processing and modification

The collagen fibril assembly is a complex process and the current understanding is largely based on in vitro experiments. The fibril-forming collagens I, II, III, V, XI spontaneously aggregate after processing of procollagens into ordered fibrillar structures in vitro, a process which has been compared to crystallization with initial nucleation and subsequent organized aggregation [38,99]. The ability for the "self-assembly" is encoded in the structure of the collagens and several models describe the mechanism for the periodic fibrillar assembly. Hydrophobic and electrostatic interactions of collagen monomers are involved in the quarter-staggered arrangement of collagen monomers, which may aggregate into five-stranded fibrils and subsequently into larger fibrils [3,99,100] (Fig. 2). The formed fibrils can be orientated differently in distinct types of tissues. In tendons, the type I collagen fibrils align parallel to each other and form bundles or fibres, whereas in the skin, the orientation is more randomly with the formation of a complex network of interlaced fibrils [38]. Furthermore, the fibril formation is influenced by the propeptides of procollagen molecules. Thus, the cleavage of the C-propeptides of type I collagen is an essential step for regulating fibril formation, but the function of the N-terminal propeptides in this process is still not fully understood and may differ between collagen types. It has been suggested that they may regulate the diameter of the forming fibrils and their removal from type I procollagen influences the regular fibril morphology [3,38].

The molecular arrangement into fibrils is additionally stabilized by the formation of covalent cross-links which finally contribute to the mechanical resilience of collagen fibrils. The hydroxylation state of telopeptide lysine residues is crucial in defining collagen cross-links. Lysine hydroxylation within the telopeptides is catalyzed by an enzyme system different from the lysyl hydroxylase responsible for helical residues. The extent of hydroxylation in the telopeptides varies between different tissues with complete hydroxylation of lysine residues in cartilage, but no detectable hydroxylation of telopeptide lysine in the skin [4]. The copper-dependent enzyme lysyl oxidase catalyzes

the formation of aldehydes from lysine and hydroxylysine residues in the telopeptides. Subsequent spontaneous reactions result in the formation of intermediate cross-links. Lysine-derived telopeptide aldehydes interact with adjacent lysine residues from adjacent molecules to form Schiff base (aldimin) cross-links, whereas the presence of hydroxylysine-derived telopeptide aldehydes allows to form more stable ketoimine bonds. During maturation of the tissue, the reducible intermediate cross-links (aldimines and ketoimines) are converted to non-reducible mature products: The Schiff bases are converted to non-reducible histidin adducts while the ketoimines react either with hydroxylysine aldehyde or a second ketoimine to form pyridinium cross-links. Alternatively, pyrrolic cross-links are formed in case of ketoimines reacting with lysyl aldehyde components [4]. Pyridinium compounds and pyrroles result in a cross-link between three collagen molecules. Most cross-links have been shown to be located at the overlap position connecting the N- or C-telopeptides with specific residues within the helical part of adjacent molecules (Fig. 2) [4].

These intermolecular cross-links are a prerequisite for the physical and mechanical properties of collagen fibrils and a stable network formation.

5. Functions of collagens beyond biomechanics

As discussed earlier, collagens serve within the body to a large extent for the maintenance of the structural integrity of tissues and organs. This is true for all parenchymal organs where they represent the major component of the ''interstitial'' matrix as well as the basement membranes. This is even more obvious for all ''connective'' tissues and in particular bone and cartilage where collagens provide the major functional backbone of the structures. Besides this, the formation of a defined pericellular microenvironment is important for the cellular integrity, as seen with collagen VI in articular cartilage, but presumably also in bone (own unpublished observation). Besides the biomechanical aspects, however, collagens are also involved in a plethora of additional functions. Specific receptors mediate the interaction with collagens, like integrins, discoidin-domain receptors, glycoprotein VI [101] or specialized proteoglycan receptors [102].

Signaling by these receptors defines adhesion, differentiation, growth, cellular reactivities as well as the survival of cells in multiple ways.

Collagens contribute to the entrapment, local storage and delivery of growth factors and cytokines and therefore play important roles during organ development, wound healing and tissue repair [1,103]. Collagen type I has been shown to bind decorin, and thereby, it might block indirectly TGF-β-action within the tissue [1]. Collagens also bind a number of other growth factors and cytokines. Thus, IGF-I and -II are bound to the collagenous matrix of bone and, therefore, bone represents a major reservoir of these growth factors within the body [104]. In bone, degradation of the collagen network by osteoclasts during bone remodeling is thought to release matrix-bound IGFs and, thus, to induce new bone formation via stimulation of osteoblastic activity in a paracrine manner. Similar effects may be active in articular cartilage and could be due to anabolic activation of chondrocytes via release of bound growth factors after cartilage matrix degradation. Type IIA collagen was recently shown to be able to bind TGFβ and BMP-2 [105]. Thus, collagens are very likely to be relevant for certain cellular reactions. This potential of collagens to bind growth factors and cytokines qualifies these molecules also as transport vehicles for therapeutic factor delivery (for review, see other chapters of this issue).

Recently, it became evident that collagens are involved in more subtle and sophisticated functions than just the architecture of extracellular matrices. Non-collagenous fragments of collagens IV, XV and XVIII have been shown to influence angiogenesis and tumorigenesis and their biological functions may not only be limited to these processes, but also influence various cellular reactivities [106–108]. Therefore, these fragments (matricryptins) attracted great interest for potential pharmaceutical uses.

6. Perspectives

Collagens are the most abundant group of organic macro-molecules in an organism. First, collagens serve important mechanical functions within the body, particularly in connective tissues. Thus, in bone, tendon, fascia, articular cartilage, etc., fibrillar colla-

gens are providing most of the biomechanical properties essential for the functioning of these organ systems. Second, collagens also exert important functions in the cellular microenvironment and are involved in the storage and release of cellular mediators, such as growth factors. All aspects mentioned above define collagens as interesting targets as well as tools of pharmacological intervention. A proper collagen matrix in terms of its composition and supramolecular organization is the target of any repair process of connective tissue whether occurring naturally, like during fracture healing or following treatment of bone non-unions after trauma, tumor-surgery or of cartilage defects (for review, see Aigner and Stöve, this issue). Finally, it should be considered that some additional features of collagens, such as biodegradability, low immunogenicity and the possibilities for large-scale isolation make them interesting compounds for a widespread industrial use in medicine, cosmetics or food industry.

Acknowledgements

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