Dynamics of Bone and Cartilage Metabolism

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Dynamics of Bone and Cartilage Metabolism

Edited by

MARKUS J. SEIBEL
Department of Medicine
Division of Endocrinology and Metabolism
University of Heidelberg Medical School
Heidelberg, Germany

SIMON P. ROBINS
Skeletal Research Unit
Rowett Research Institute
Aberdeen, United Kingdom

JOHN P. BILEZIKIAN
Departments of Medicine and Pharmacology
Division of Endocrinology
College of Physicians and Surgeons
Columbia University
New York, New York

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Contributors

Kristina Åkesson
Associate Professor, Department of Orthopedics, Malmö University Hospital, 205 02 Malmö, Sweden

Sari L. Alatalo
Finnish Red Cross Blood Service, Helsinki, Finland

Susan J. Allison
Postgraduate Scholar, Bone Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Sydney NSW 2010, Australia

Ziyad Al Aly
Division of Nephrology, Saint Louis University School of Medicine, St. Louis, Missouri, USA

Paul A. Baldock
Senior Research Officer, Bone Research Program, Garvan Institute of Medical Research,384 Victoria Street, Sydney NSW 2010, Australia

John P. Bilezikian
Departments of Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, NY

John P. Bilezikian
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA
Departments of Medicine and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, USA

Neil Binkley
University of Wisconsin, Madison, Wisconsin, USA

Jean-Jacques Body
Dept of Internal Medicine and Endocrinology/ Bone Diseases Clinic, Institut J. Bordet, Univ. Libre de Bruxelles, Brussels, Belgium

Jean-Philippe Bonjour
Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Department of Rehabilitation and Geriatrics, University Hospitals, CH - 1211 Geneva 14 (Switzerland)

Adele L. Boskey
Starr Chair in Mineralized Tissue Research, Hospital for Special Surgery, New York, NY 10021 and Weill Medical College and Graduate School of Medical Sciences of Cornell University, New York, NY 10021

Roger Bouillon
Laboratorium for Experimental Medicine and Endocrinology, K. U. Leuven, Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium

Kim Brixen
Department of Endocrinology, Odense University Hospital, DK-5000 Odense C, Denmark

Peter Bruckner
Department of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany

Geert Carmeliet
Laboratorium for Experimental Medicine and Endocrinology, K. U. Leuven, Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium

Ian M Clark
School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

Peter Croucher
Academic Unit of Bone Biology, University of Sheffield Medical School, Sheffield S10 2RX, United Kingdom

Pierre D. Delmas
Professor of Medicine, Université Claude Bernard, Lyon, France and Director INSERM Research Unit 403, Lyon, France

David W. Dempster
Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, USA

Jean-Pierre Devogelaer
Department of Rheumatology, Saint Luc University Hospital, Université Catholique de Louvain, 1200 Brussels, Belgium

Marc K. Drezner
Professor of Medicine, University of Wisconsin, Madison, Wisconsin, USA

Richard Eastell
From the Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, UK

Guy Eelen
Laboratorium for Experimental Medicine and Endocrinology, K. U. Leuven, Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium

Erik Fink Eriksen
Novartis Pharma, Basel, Switzerland

Lorraine A. Fitzpatrick
Global Development, Amgen, Thousand Oaks, CA

Ghada El-Hajj Fuleihan
Calcium Metabolism and Osteoporosis Program, American University of Beirut-Medical Center, Beirut, Lebanon

Edith M. Gardiner
Associate Professor, School of Medicine, The University of Queensland, Head, Skeletal Biology Unit, Centre for Diabetes & Endocrine Research, Ground Floor, C Wing, Bldg 1, Princess Alexandra Hospital, Ipswich Road, Brisbane QLD 4102, Australia

Patrick Garnero
Research Scientist, INSERM research unit 403 and Vice-President Synarc Molecular Marker Division, Lyon, France

Renate E. Gay
Research Scientist, INSERM research unit 403 and Vice-President Synarc Molecular Marker Division, Lyon, France

Steffen Gay
Research Scientist, INSERM research unit 403 and Vice-President Synarc Molecular Marker Division, Lyon, France

Francis H. Glorieux
Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Québec, Canada H3G 1A6

Mary B. Goldring
Medical Center, Harvard Medical School, Boston, MA; New England Baptist Bone and Joint Institute, Boston, MA 02215

Steven R. Goldring
Department of Medicine, Rheumatology Division, Beth Israel Deaconess

David Goltzman
Calcium Research Laboratory, Department of Medicine, Royal Victoria Hospital, McGill University, Montreal, H3A 1A1, Canada

Esther A. González
Division of Nephrology, Saint Louis University School of Medicine, St. Louis, Missouri, USA

Andreas Grauer
Procter & Gamble Pharmaceuticals Mason, OH, USA

Caren M. Gundberg
Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut 06510

Tim Hardingham
Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom

John R. Harrison
Division of Orthodontics, University of Connecticut Health Center, Farmington, CT 06030

Dick Heinegård
Departments of Experimental Medical Science and Clinical Science, BMC plan C12, SE-22184, Lund, Sweden

M. H. Helfrich
Department of Medicine and Therapeutics, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, United Kingdom

Herbert Herzog
Adjunct Professor, Faculty of Medicine, The University of New South Wales, Principal Research Fellow, Head Obesity and Energy Homeostasis Research Group, Director Neurobiology Program, Garvan Institute of Medical Research, 384 Victoria Street, Sydney NSW 2010, Australia

M. A. Horton
Bone and Mineral Centre, Department of Medicine, The Rayne Institute, London WC1E 6JJ, United Kingdom

Philippa Hulley
Botnar Research Centre, Institute of Musculoskeletal Sciences, University of Oxford, Oxford OX3 7LD, United Kingdom

Harald Jüppner
Endocrine and Pediatric Nephrology Units, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Helena Kaija
Research Center for Molecular Endocrinology, University of Oulu, Finland

S. Khosla
Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Department of Rehabilitation and Geriatrics, University Hospitals, CH - 1211 Geneva 14 (Switzerland)

Marius E. Kraenzlin
Division of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

Barbara E. Kream
Departments of Medicine and Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Ct 06030

Carolina A. Moreira Kulak
Department of Endocrinology, Federal University of Parana, Hospital de Clinicas, Curitiba, Brazil
Division of Endocrinology and Metabology of Hospital de Clinicas, Federal university of Parana (SEMPR), Curitiba-/Brazil

Johannes P.T.M. van Leeuwen
Department of Epidemiology & Biostatistics, Erasmus Medical Centre, Rotterdam, The Netherlands

Gary L. Lensmeyer
University of Wisconsin, Madison, Wisconsin, USA

Jane B. Lian
University of Massachusetts Medical School, Department of Cell Biology, 55 Lake Avenue North, Worcester, MA 01655, USA

Joseph A. Lorenzo
Director, Bone Biology Research, Professor of Medicine, University of Connecticut Health Center, 263 Farmington Avenue, MC 1317, Farmington, CT 06030–1317.

Pilar Lorenzo
Departments of Experimental Medical Science and Clinical Science, BMC plan C12, SE-22184, Lund, Sweden

Christa Maes
Laboratorium for Experimental Medicine and Endocrinology, K. U. Leuven, Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium

Daniel-Henri Manicourt
Laboratoire de Chimie Physiologique (Metabolic Research Group, Connective Tissue Section), Christian de Duve Institute of Cellular Pathology and Department of Rheumatology, Saint Luc University Hospital, Université Catholique de Louvain, 1200 Brussels, Belgium

Klaus von der Mark
Dept. of Experimental Medicine and Connective Tissue Research, Friedrich-Alexander, University of Erlangen Nuremberg, Germany

Kevin J. Martin
Division of Nephrology, Saint Louis University School of Medicine, St. Louis, Missouri, USA

T. John Martin
St. Vincent's Institute of Medical Research, 9 Princes Street, Fitzroy 3065, Australia

Christian Meier
Endokrinologische Praxis & Labor,
University Hospital Basel, Switzerland

Joyce B.J. van Meurs
Department of Epidemiology & Biostatistics, Erasmus Medical Centre, Rotterdam, The Netherlands

José Luis Millán
The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037-1005, USA

David G. Monroe
Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN

Jean E. Mulder
Department of Medicine, Brigham and Women's Hospital, Harvard University, Boston, MA 02115

Gillian Murphy
Dept of Oncology, Cambridge University, Cambridge CB2 2XY, UK

Kim E. Naylor
From the Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, UK

Michel Neidhart
Research Scientist, INSERM research unit 403 and Vice-President Synarc Molecular Marker Division, Lyon, France

Tuan V. Nguyen
Bone and Mineral Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital and University of NSW Research Program
University of New South Wales, Sydney, Australia

Satoru K. Nishimoto
Department of Molecular Sciences, The University of Tennessee College of Medicine, Memphis, Tennesse 38163

Lila O.T. Patrikainen
Research Center for Molecular Endocrinology, University of Oulu, Finland

Huibert A.P. Pols
Department of Endocrinology, Laboratory Group, Im Breitspiel 15, 69126 Heidelberg, Germany

Lawrence G. Raisz
Interim Director, Musculoskeletal Institute, Board of Trustees Distinguished Professor of Medicine, University of Connecticut Health Center, 263 Farmington Avenue, MC-3805, Farmington, CT 06030

Stuart Ralston
Rheumatic Diseases Unit, University of Edinburgh, Edinburgh, UK

Frank Rauch
Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Québec, Canada H3G 1A6

Ian R. Reid
Department of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand

Juha Risteli
Department of Clinical Chemistry, FI-90014 University of Oulu, Oulu, Finland

Leila Risteli
Department of Clinical Chemistry, FI-90014 University of Oulu, Oulu, Finland

Fernando Rivadeneira
Department of Epidemiology & Biostatistics, Erasmus Medical Centre, Rotterdam, The Netherlands

René Rizzoli
Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Department of Rehabilitation and Geriatrics, University Hospitals, CH - 1211 Geneva 14 (Switzerland)

Simon P. Robins
Matrix Biochemistry Group, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB

Clifford J. Rosen
Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, 900 Broadway, Bldg #2, Bangor, Maine 04401 USA

Mishaela R. Rubin
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Graham Russell
Botnar Research Centre, Institute of Musculoskeletal Sciences, University of Oxford, Oxford OX3 7LD, United Kingdom

Tore Saxne
Departments of Experimental Medical Science and Clinical Science, BMC plan C12, SE-22184, Lund, Sweden

Heinrich Schmidt-Gayk
Department of Endocrinology, Laboratory Group, Im Breitspiel 15, 69126 Heidelberg, Germany

Ego Seeman
Dept of Endocrinology and Medicine, Austin Hospital, University of Melbourne, Melbourne, Australia.

Markus J. Seibel
Bone Research Program, ANZAC Research Institute, University of Sydney and Concord Hospital, Sydney, Australia

Elizabeth Shane
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Shonni J. Silverberg
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, USA

Peter A. Simkin
Professor of Medicine, Adjunct Professor of Orthopaedics, University of Washington

Natalie A. Sims
University of Melbourne, Department of Melbourne, St. Vincent's Health, 41 Victoria Pde, Fitzroy 3065, Australia

Ethel Siris
Department of Medicine, Columbia University College of P&S, New York, NY, USA

Thomas C. Spelsberg
Professor of Biochemistry, Mayo Clinic College of Medicine, Rochester, MN

Gary S. Stein
University of Massachusetts Medical School, Department of Cell Biology, 55 Lake Avenue North, Worcester, MA 01655, USA

Eugene J.-M. A. Thonar
Departments of Biochemistry, Internal Medicine and Orthopedic Surgery, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, 60612 USA

André G. Uitterlinden
Department of Internal Medicine,

H. Kalervo Väänänen
Institute of Biomedicine, Department of Anatomy, University of Turku, Turku, Finland

Annemieke Verstuyf
Laboratorium for Experimental Medicine and Endocrinology, K. U. Leuven, Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium

Pirkko T. Vihko
Department of Biological and Environmental Sciences, Division of Biochemistry, University of Helsinki, Finland

Michael P. Whyte
Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children; and Division of Bone and Mineral Diseases, Washington University School of Medicine At Barnes-Jewish Hospital; St. Louis, Missouri

Hua Zhou
Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, USA

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Preface to the Second Edition

The first edition of "Dynamics of Bone & Cartilage Metabolism", published at the very end of the last century, in 1999, was successfully received by biomedical scientists in the bone field and by clinicians throughout the world. Since the first edition was published, research in bone and cartilage metabolism has progressed at a rapid pace leading to new insights into basic science as well as new ways in which markers of bone and cartilage metabolism can be used clinically.

This second edition of "Dynamics of Bone & Cartilage Metabolism" incorporates these advances while maintaining the general structure of the first edition. It is a thorough update with all chapters either extensively revised or completely rewritten. To reflect the changing climate of knowledge, twelve new chapters have been added that, we believe, greatly enhance the substance and completeness of the book. The topics of these new chapters include: "Acid Phosphatases", "Bone Structure, Architecture and Strength", "Signalling Mechanisms in Bone", "The Central Control of Bone Remodelling", "Transgenic Models of Bone Disease", "Models of Cartilage Metabolism and Disease", "Measurement of Parathyroid Hormone", "Measurement of Vitamin D", "Variability of Bone Markers", "Monitoring of Anabolic Treatment", "Monitoring of Anti-resorptive Treatment" and "Osteogenesis Imperfecta". These new chapters add greatly to updated chapters and together provide a complete repository of information on this subject.

We are grateful to all authors for their efforts to deliver their new or revised chapters within the time constraints, always a challenge. We are also grateful to the excellent staff at Elsevier-Academic Press, Tari Broderick, Karen Dempsey and Renske van Dijk, who helped us so enthusiastically throughout the preparation of this new edition of *Dynamics of Bone and Cartilage Metabolism.*

MARKUS SEIBEL, Sydney

SIMON ROBINS, Aberdeen

JOHN BILEZIKIAN, New York

June 2006

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PART I

Components of the Organic Extracellular Matrix of Bone and Cartilage

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CHAPTER I

Structure, Biosynthesis and Gene Regulation of Collagens in Cartilage and Bone

KLAUS VON DER MARK Department of Experimental Medicine and Connective Tissue Research, Friedrich-Alexander, University of Erlangen Nuremberg, Germany

I. Introduction
II. The Collagen Families
III. Bone Collagens
IV. Cartilage Collagens
V. Collagen Biosynthesis
VI. Collagen Genes and Transcriptional Regulation
VII. Factors Regulating Collagen Biosynthesis
VIII. Conclusions
References

I. INTRODUCTION

Collagens provide the structural framework of bones and cartilages and hold responsibility for shape and most of the biomechanical properties such as resistance to pressure, torsion, and tension [1]. In vertebrates, 27 genetically distinct collagen types with rather diverse structural and biochemical features have been identified, but only about half of them are represented in cartilage and bone [2–4] (Table I). Their specific functions in the tissues are only partially known.

In cartilage and bone, fibril-forming collagens are dominant: the bone matrix consists basically of two collagen types, about 95% type I and 5% type V collagen which are assembled into heterofibrils [5]. Similarly, the backbone of all cartilages is made of types II/XI collagen heterofibrils which are decorated with so-called FACIT collagen types IX, XII, or XIV (see below) [3, 4, 6, 7]. These fibrils are interwoven with a microfibrillar mesh made of type VI collagen which may provide additional elasticity [8–11]. In addition, cartilage contains minor amounts of other collagen types depending on the cartilage type and location (see below).

The basic function of collagens in cartilage and bone is to provide the structural scaffold to tissues into which minerals, proteoglycans, and glycoproteins can be firmly incorporated, thus being responsible for the unique physiological and mechanical properties of these tissues. But on top of biomechanical functions, collagens play an important role in all tissues, including cartilage and bone as biological substrates for cell adherence. Essential cell biological functions such as proliferation, cytoskeletal organization, migration, differentiation, and apoptosis are regulated by collagens, mediated by transmembrane receptors of the integrin and syndecan families [12].

Since collagens provide the major organic component with 90% of the dry mass in bone, or 60% in cartilage, respectively, it is obvious that defects in structure,

TABLE I. Collagens in Cartilage and Bone

Type	Subunits	Molecular forms	Tissue distribution	Characteristic features
			Fibril-forming collagens	
I	α1(I) α2(I)	$\alpha1(I)_2\,\alpha2$	Bone, dermis, tendon, ligaments cornea, most other tissues	Forms fibers of high tensile strength; most abundant collagen
I	α1(I)	$[\alpha1(I)]_3$	Dermis, dentin	Rare form
II	α1(II)	$[\alpha1(II)]_3$	Cartilage, notochord, vitreous body embryonic epithelia, retina	Major cartilage collagen; forms heterofibrils with Col IX + XI
III	α1(III)	$[\alpha1(III)]_3$	Reticular fibers of most tissues (lung, liver, dermis, spleen, vessel wall, etc.)	Often in mixed fibrils with type I collagen; present in reticular fibers and elastic tissues; cystine bridges in triple helix
V	α1(V) α2(V) α3(V)	$[\alpha1(V)]_3$ $[\alpha1(V)]_2\,\alpha3(V)$	*In vitro:* hamster lung cell cultures lung, cornea, bone, fetal membranes; together with Col I	Propeptide partially retained in the fibrils; forms hetero-fibrils with type I collagen controls fibril diameter
XI	α1(XI) α2(XI) α3(XI)	[α1(XI) α2(XI) α3(XI)] $[\alpha1(XI)]_2\,\alpha2(V)$	Cartilage, vitreous body Bone	Homologous to Col V; nucleates and controls cartilage coll. fibril formation; α3(XI) same gene as for α1(II)
XXIV	α1(XXIV)	n.d.	Bone, eye	Similar to type V collagen, contains TSP motif in N-propeptide
XXVII	α1(XXVII)	n.d.	Cartilage; eye and ear	*Col27a1* gene 156 kb, 61 exons
			Microfibrillar collagen	
VI	α1(VI) α2(VI) α3(VI)	[α1(VI) α2(VI) α3(VI)]	Widespread, in cartilage (pericellular), intervert, disk dermis, placenta, lung vessel wall	Contains vWF and Kunitz type protein inhibitor domains; forms beaded filaments; highly disulfide crosslinked
			Network forming, short chain collagens	
X	α1(X)	$[\alpha1(X)]_3$	Hypertrophic cartilage	Strong inter- and intramolecular interactions between NC1-domains Mutations in Col X-NC-1 →SMCD
			FACIT collagens	
IX	α1(IX) α2(IX) α3(IX)	[α1(IX) α2(IX) α3(IX)]	Cartilage, vitreous humor Splice variant without NC-4 domain in cornea	Covalently linked to type II collagen fibrils; NC4 domain projects into cartilage matrix; contains chondroitin sulfate
XII	α1(XII)	$[\alpha1(XII)]_3$	Perichondrium, ligaments, tendon	Large cruciform shaped NC3 domain; associated with type I collagen fibrils
XIV	α1(XIV)	$[\alpha1(XIV)]_3$	Cartilage, dermis, tendon, vessel wall, placenta, lung, liver	Associated with type I collagen
XVI	α1(XVI)	$[\alpha1(XVI)]_3$	Cartilage (territorial matrix) papillary dermis	Integrates into discrete Col II/XI fibrils
XX	α1(XX)		Cornea; sternal cartilage	Less FN III repeats than Col XIV
XXI	α1(XXI)		Blood vessel wall	TSP and vWFA domain, but no FNIII rep.
XXII	α1(XXII)		Myotendinous junction, articular cartilage surface	Present only in tissue junctions

biosynthesis, assembly, or turnover of collagens generally will lead to severe diseases such as osteoporosis, osteoarthritis, chondrodysplasias, or osteogenesis imperfecta. In order to understand the specific functions of the various collagens in cartilage and bone and the reasons for their failure in connective tissue diseases, it is necessary to have a close look at the specific structural and biochemical features of the collagens. An overview on the structural and functional features of cartilage and bone collagens, and their interactions with other matrix proteins and cell receptors, will therefore be given in the first part of this chapter.

In order to understand the dynamics of collagen metabolism in bone and cartilage, it is helpful to gain insight into the various levels of transcriptional and translational regulation of collagen biosynthesis and turnover. Therefore, in the second part of this chapter, the various steps of collagen biosynthesis and post-translational modifications will be summarized, and an overview is given on the structure of the collagen genes and their *cis*-acting regulatory element. How these are regulated by growth factors, cytokines, hormones, and transcription factors is one of the current challenges in understanding dynamics of connective tissue turnover and homeostasis.

The scope of this chapter does not permit a detailed and complete presentation of our current knowledge on structure, biosynthesis, and regulation of collagens in bone

and cartilage. Fortunately, a number of excellent review articles and book chapters are available, such as the recent, most comprehensive book chapter on collagens by Kielty and Grant [2] and many other articles dealing with structure and function of collagen types [6, 13–16], with collagen gene [17–19], collagen biosynthesis and regulation [20–23], collagen degradation [24, 25], and collagen-related diseases [26–29]. No further information can be given here on collagens which do not play a major role in cartilage and bone such as types IV, VII, VIII, and XIII–XIX. Aspects of extracellular assembly to supramolecular structures, in particular fibril formation and cross-link formation, will be dealt with in Chapters 2, 11, and 20. Collagen degradation by proteases will be covered in Chapter 10 on matrix proteases.

II. THE COLLAGEN FAMILIES

Up to 2004, at least 27 genetically distinct collagen types had been identified in mammals, encyphered by 42 genes coding for their subunits [2, 6, 15] (Table I). Based on molecular structure and sequence homology, collagens have been grouped into seven or eight different families, including the fibril-forming collagens, FACIT collagens, microfibrillar collagens, network-forming collagens, transmembrane collagens, multiplexins, and others (see Table I). Since the first edition of this book seven new collagen types have been discovered, partially by screening sequence data banks for homology with collagen and procollagen peptide sequences [30–37]. Four of these new collagens (types XX, XXI, XXII, and XXIV) were found also in cartilage, but their functions are not yet known.

All collagens consist of one or several collagenous, triple-helical domains, flanked or interrupted by noncollagenous domains which are largely removed by proteolytic processing in fibrillar collagen type I, II, and III procollagen. They are retained, however, in the mature molecule in most non-fibril-forming collagens.

A. The Collagen Triple Helix

The key feature of all collagens is the triple helix, a coiled-coil structure in the form of a right-handed helix of 1.5 nm diameter, composed of three polypeptide chains (α-chains) [38–40] (Fig. 1). A structural requirement for the assembly of polypeptide chains into a collagen triple helix is the occupation of every third position by a glycine residue, resulting in the $(Gly\text{-}X\text{-}Y)_n$ repeat structure characterizing all collagens. The α-chains form a stretched, left-handed helix with a pitch of 18 amino acid residues per turn [41] and assemble around a central axis in a manner allowing all glycine residues to be positioned in the center of the triple helix. The more bulky side chains of the other amino acids in the X and Y position occupy the outer positions, where they are available for lateral interactions with adjacent collagen molecules to form fibrils. Another typical feature of the collagen triple helix is the high content of proline and hydroxyproline (ca. 20%). The hydroxyl groups of 4-hydroxy-proline are essential for the formation of intramolecular hydrogen bonds and thus critically determine the thermal stability of each collagen triple helix [40, 42]. The melting temperature of the type I collagen triple helix is 39°C at neutral pH, but can be considerably higher, e.g. 46°C in chicken type X collagen [43], or 65°C in the aminoterminal 7S domain of type IV collagen [44]. Triple helical domains vary considerably in their length: in fibril-forming collagens they span 300 nm, corresponding to 1000 amino acid residues per processed α-chain, while in other collagens, such as the multiplexins, they may include only nine triplets. Interruptions of the Gly-X-Y-Gly-X-Y- structure by one amino acid residue causes flexibility in the triple helix and renders the helix susceptible to proteolytic attack, e.g. in collagen type IV [45]. The native or denatured state of a triple helix may be measured by optical rotation, circular dichroism or resistance to proteases like pepsin, trypsin, or chymotrypsin [46]. For example, native type I collagen molecules have an optical rotation of $\varepsilon = -1000°$ at 405 nm, which drops

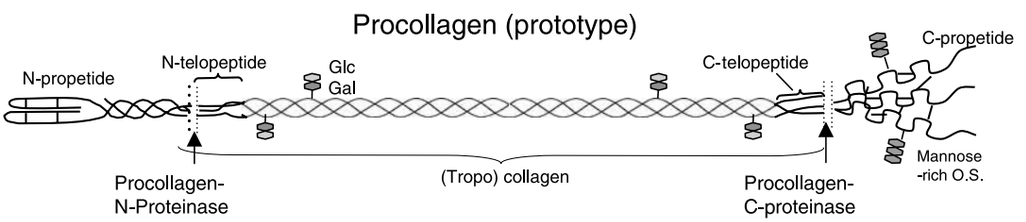

FIGURE 1 Type I procollagen as a prototype of fibril-forming collagens. In types I, II, and III procollagens N- and C-terminal propeptides are removed after secretion by specific proteases, in types XI and V the N-propeptides are larger (see Fig. 3) and only partially cleaved. The C-propeptides contain AsN-linked mannose-rich oligosaccharides, while the triple helical part contains only hydroxylysine-linked glucosyl-galactosyl disaccharides or monosaccharides.

to –336° after denaturation. The resistance of the native triple helix to most proteases has been the basis for almost all biochemical isolation procedures of collagens in the past, using pepsin to destroy non-collagenous proteins while leaving the collagen triple helices intact.

Triple helical collagenous domains are also found in proteins such as C1q, acetylcholine esterase and MARCO, a macrophage scavenger receptor [47, 48].

B. Noncollagenous Domains

In the various collagens the triple helical domains are flanked or interrupted by noncollagenous domains. While the triple helix is a highly conserved structural protein element like the α-helix or the β-pleated sheet, there is a wide structural and functional diversity among the noncollagenous domains of the different collagen families, often bearing essential functions specific for each collagen.

Fibril-forming collagens are synthesized as procollagens with a triple helix of 300 nm length, which is flanked by noncollagenous propeptides (NC-domains) at both ends [2, 49]. The globular C-propeptides, consisting of about 250 amino acid residues per α-chain, are all homologous and serve as a nucleation site for chain assembly and triple helix formation, while the N-propeptides regulate the fibril size when retained in the molecule [50, 51]. These procollagen peptides are largely removed by specific proteases after secretion, a prerequisite for fibril formation [52]. The C-propeptide of type II collagen remains in cartilage after cleavage from the procollagen molecule as a stable, hydroxyapatite-binding molecule ("chondrocalcin") and may participate in cartilage calcification [53]. The C-terminal NC-1 domain of type X collagen, a highly compact and stable, bell-shaped trimer, also binds calcium and is highly homologous to TNFα [54], but does not bind to the TNFα receptor; its role is rather structural, serving as a nucleation site for triple helix assembly as well as for network assembly [55, 56].

In "FACIT" collagens (fibril-associated collagens with interrupted triple helices) [57], which include collagen types IX, XII, XIV, XVI, XIX, XX, XXI, and XXII, the collagenous domains are interrupted by two or three noncollagenous domains, while the aminoterminal domains form large, cross-shaped entities anchored in the extracellular matrix. In the more recently discovered "multiplexins" (collagens with multiple triple helix interruptions, [58] see Table I) the collagenous domains are short and separated by nine or ten noncollagenous domains. Despite the differences between fibril-forming and FACIT collagens, there is also sequence homology among the N-terminal NC-domains of IX, XI, XII, and XVI collagens. Many noncollagenous domains, e.g. in collagen VI and XI, contain thrombospondin- and von Willebrand-factor-like domains or fibronectin-type III repeats [59].

In the non-fibril-forming collagens, the noncollagenous domains often show more specific and important structural and functional features than the triple helical domains. Thus, the C-propeptide of type XVIII collagen is retained after cleavage as a protein with antiangiogenic properties (endostatin) [60].

C. Structural and Functional Diversity of Collagens

There is considerable complexity and diversity in the structure of the different collagen types, their splice variants, their triple helical and nontriple helical domains, and their assembly into extracellular matrix structures: fibrils, flexible meshworks, hexagonal sheets, beaded filaments, anchoring fibrils and perhaps other, yet unknown, structures [15].

The fibril-forming collagens represent with seven members (including the recently discovered collagen XXIV and XVII) and about 90% of the total collagens, the most abundant and widespread family of collagens in vertebrates. Type IV collagens with a more flexible triple helix assemble into supercoiled chicken-wire like meshes that are restricted to basement membranes. Types VIII and X collagens are short-chain collagens which form hexagonal sheets. Type VI collagen is highly disulfide cross-linked and assembles into a meshwork of beaded filaments interwoven with collagen fibrils. Collagens IX, XII, and XIV associate as single molecules with collagen fibrils (FACIT-collagens) [3, 7], while others (Col XIII and XVII) span cell membranes [61–63]. Little is known on the extracellular architecture of the more recently discovered collagens, except for collagens XXIV and XXVII, which have all the features of fibril-forming collagens [37].

Owing to their tensile strength and torsional stability, the major function of fibril-forming collagens is to support tissue architecture and stability. Despite structural similarity, however, the fibrillar collagen types I, II, and III have different, specific functions in different tissues, different immunological properties and show specific interactions with different cell types. For example, replacement of type II collagen by the similar type I collagen in cartilage, e.g. in joint repair by fibrous cartilage, causes severe loss of cartilage-specific features of the tissue. Initial concepts of specific collagen functions were mostly derived from their distribution in tissues and from *in vitro* experiments, i.e. from cell and organ culture studies. Recently, there is ample evidence for specific cell biological functions of

fibrillar collagens, such as types I and II collagen, in serving as substrates for cell adhesion, proliferation, migration, and differentiation, mediated by β1 integrins [12, 64–67].

More recently, natural and artificially introduced mutations in human and animal collagen genes, and the inactivation of collagen genes by homologous recombination in mice ("knockout mice") [68–70], have provided valuable information on the role of some collagens in cartilage and bone. Surprising results of gene knockout experiments in mice or from human mutations have often caused a revision of common opinions on the function of various collagens; thus, after inactivation of the type II collagen gene *Col2a1* in mice, rather normal development of long bones and of the eye was observed, although early expression of *Col2a1* in embryonic corneal and retinal epithelia [71–73] and several *in vitro* studies had strongly suggested a critical role of collagen in early epithelial–mesenchymal interactions [71, 74]. The lack of type II collagen in the notochord of the Col2a1$^{-/-}$ mice did not prevent somite differentiation as expected [75], but the resorption of the notochord during development of the spine [69]. More and valid information on the function of individual collagens and their domains have been obtained from targeted mutations in distinct domains and tissue-specific inactivation (conditional knockout) of collagen genes.

III. BONE COLLAGENS

The organic mass of the bone matrix comprises about 90% of type I and 5% of type V collagen [76], the remainder being bone-specific phospho- and glycoproteins such as osteopontin, bone sialoprotein, osteocalcin, osteonectin, and others. In bone, type I and V collagen assemble into a quarter-staggered heterofibrils with diameters between 25 and 400 nm (Fig. 2). Fibrils thicker than 50 nm show a characteristic banding pattern in the electron microscope with a periodicity of 65–67 nm (D-period) [16, 77, 78]. Adachi and Hayashi [79] have shown that inclusion of type V collagen controls the fibril diameter of type I collagen fibrils; in embryonic chick cornea, for example, a content of 20% type V collagen limits the fibril diameter to 25 nm [80]. In contrast, collagen fibrils in bone with a content of ca. 5% type V collagen reach diameters of 400 nm or more [76, 81]. Embedded in hydroxyapatite crystals and various bone-specific phosphoproteins and glycoproteins and SLRPs (small leucine-rich proteoglycans) such as osteoadherin (see Chapter 3), type I/V heterofibrils reveal unmatched biomechanical properties concerning load bearing, tensile strength, and torsional resistance. They serve also as a nucleation site for hydroxyapatite crystals [82] (see Chapter 12).

In the osteons of compact bone, collagen fibrils seem to run parallel in two nearly orthogonal directions, forming twisted, nearly rectangular plywood-like layers [83].

As the assembly of collagen molecules into fibrils will be dealt with in detail in Chapter 2, here the structure of the bone collagen molecules, their biosynthesis and regulation, are focused on.

A. Type I Collagen

1. MOLECULAR STRUCTURE AND TISSUE DISTRIBUTION

Type I collagen is the most abundant, longest-known and best-studied collagen in vertebrates. It forms 90% of the organic mass of bone and tendon and is the major collagen of skin, ligaments, cornea, and many interstitial

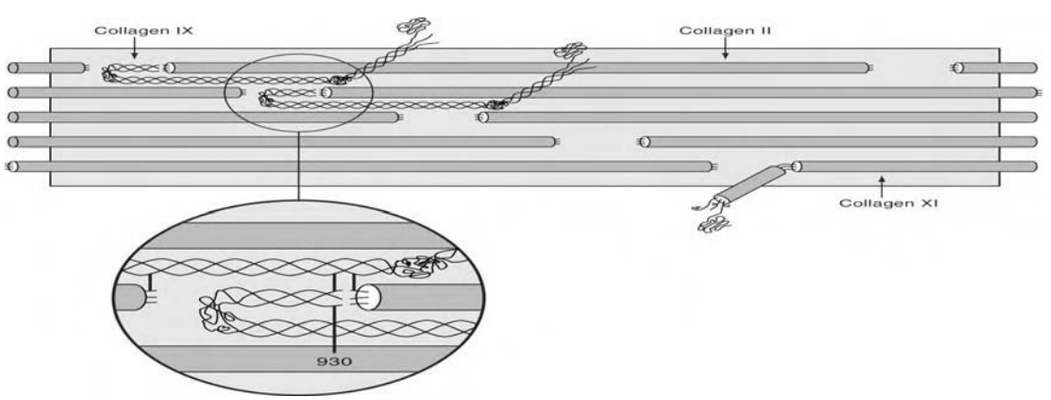

FIGURE 2 Packing of types II, XI, and IX collagen in the cartilage collagen heterofibril, showing the location of the covalent cross-links between type II and IX collagen. The globular NC4 domains of type IX collagen reach out of the fibril. (From: D. Eyre (2001) Collagen of articular cartilage. *Arthritis Res*. **4,** 30–35, with kind permission by BioMed Central, Ltd.)

connective tissues. Much of our information on biochemical and biophysical properties, cross-linking, and biosynthesis of collagens is based on research on this collagen, but may be applied to other collagens. The human type I procollagen is made of 2 proα1 chains of 1464 amino acid residues [84] and a somewhat shorter pro α2 subunit (1366 amino acid residues) (Fig. 1) [85]. It is synthesized in large quantities by fibroblasts, osteoblasts [86], and odontoblasts [87], and to a lesser extent by nearly all other tissue cells [12].

Although purified type I collagen can be reconstituted to crossbanded fibrils *in vitro, in vivo* type I collagen is always incorporated into heterofibrils containing either type III collagen, e.g. in skin and reticular fibers [88], or type V collagen in bone, tendon cornea and other tissues [76, 89] or both. Type I/III collagen heterofibrils are a constituent of reticular fibers of most parenchymal tissues such as lung, kidney, liver, muscle, or spleen, with the exception of hyaline cartilage, brain, and vitreous humor [90].

The key role of type I collagen in bone is most evident from mutations in the human type I collagen genes *COL1A1* and *COL1A2* as the cause of osteogenesis imperfecta (OI), a group of hereditary disorders characterized by a decrease in bone mass, enhanced bone fragility and multiple fractures (see also Chapter 54; for reviews see [28, 91]. The severity and progress of the disease is rather variable and ranges from mild forms to more severe and lethal forms. The mode of inheritance of the OI is in most cases autosomal dominant. More than 160 different mutations have been reported in the *COL1A1* and *COL1A2* genes, located on chromosomes 17q21.3 and 7q21.3–q22, respectively [28]. Most mutations affect glycine residues, leading to impaired triple helix formations, even in heterozygotes, but also exon skipping, frameshift mutations, RNA splicing mutations and basepair deletions or insertions have been identified. Generally it is difficult to predict the severity of the phenotype from the type of mutation. The general rule is that apparently mild mutations affecting the stability of the triple helix, owing to a glycine substitution, result in a more severe phenotype than entire exon deletions, allowing the formation of intact, although shortened, triple helices. The reason is that one affected α chain with a triple helical interruption may exert a dominant negative effect and impair seven type I collagen molecules. Furthermore, impaired triple helix formation in the rough endoplasmic reticulum leads to over-hydroxylation of proline and lysine residues [92, 93]. Premature chain terminations or deletions of exons in one COL1A1 allele which do not affect triple helix assembly may cause haplo-insufficiency, but will still allow the formation of intact collagen I molecules from the unaffected allele [28, 91].

2. PHYSIOLOGICAL FUNCTIONS

Besides its biomechanical properties, type I collagen is important as adhesive substrate for many cells and plays a major role in tissue and organ development, in cell migration, proliferation and differentiation, in wound healing, tissue remodeling, and hemostasis [12, 74]. For example, *in vitro* studies have shown that many epithelial and endothelial cells acquire a polar cell shape and develop a luminal structure when cultured in a three-dimensional hydrated collagen lattice [94]. Cells recognize native type I collagen via $\alpha1\beta1$, $\alpha2\beta1$, and $\alpha11\beta1$ integrins [65, 95] which are transmembrane receptors and confer signals from the extracellular matrix to intracellular signal cascades and to the actin cytoskeleton [96–98]. Furthermore, $\alpha11\beta1$ integrin is able to organize the assembly of extracellular type I collagen molecules into fibrils [99]. Thus, reconstituted hydrated lattices consisting of native type I collagen fibrils are widely used for cell and tissue culture purposes, allowing cells to migrate, proliferate, and differentiate in a native, three-dimensional environment. Similarly, freeze-dried collagen sponges find wide applications in surgery and tissue engineering as scaffolds for wound and tissue repair by supporting adhesion and invasion of connective tissue cells [100–102].

Valuable and important information on the role of type I collagen has been gained from the MOV13 mouse strain, in which the expression of α1(I) chains is blocked owing to an insertion of the MULV Moloney virus into the first intron [103, 104]. Homozygous embryos die at day 13.5 owing to vessel rupture, but early development of organs is normal in the absence of type I collagen. Organ culture of salivary glands or lung buds showed that branching morphogenesis is also normal in the absence of type I collagen [105], possibly owing to a supplementing effect by type III collagen. Interestingly, in organ culture of tooth and bone anlagen α1(I) collagen is expressed despite the insertion of the Moloney virus in the *Col1a1* gene [106, 107]. This observation led to the discovery of a bone-specific control of *Col1a1* transcription starting at a site which differs from the transcriptional control in fibroblasts (see Section VI).

B. Type V Collagen

Type V and XI collagen are closely related in structural and evolutionary terms and have therefore been grouped into a clade of fibril-forming collagens with similar biochemical properties and similar functions in the organism. Type V collagen usually co-distributes with type I collagen in bone, corneal stroma, interstitial matrix of smooth muscle, skeletal muscle, liver, lung, and placenta [80, 108], while type XI

collagen is attributed to cartilage collagen. Both collagens precipitate in their pepsin-treated form between 0.8 and 1.2 M NaCl at acidic pH, a feature which was decisive for their first discovery and separation from the dominant type I or type II collagens, respectively [114, 109]. Five of the pro-α-chains of collagen V and XI are further characterized by large amino terminal noncollagenous domains, which are partially retained in the fibrils [50]. The 400 amino acid residue globular domains of α1, α2, α3 (V), and α1 and α2(XI), located between signal peptide and the short triple helix of the N-propeptide, are about twice as large as the corresponding cysteine-rich region in α1(I), α1(II), and α1(III) (Fig. 2). They contain a proline/arginine rich domain (PARP domain) which is similar in α1(V), α2(V), α1(XI), and α2(XI), as well as in the N-terminal domain of collagen XII [110]. The domains are processed only partially after secretion, leaving stubs of 70–100 kDa in the fibril [50, 111] where they are critical for controlling fibril assembly and growth (see below). Unusual is the high content of tyrosine sulfate in the N-propeptide of α1(V) and α2(V) collagen chains [112]. With 40% of the tyrosine residues being O-sulfated, a strong regulating interaction with the more basic triple-helical part is likely to stabilize the fibrillar complex. In contrast to types I, II, and III collagen, the triple helical parts of types V and XI collagen are resistant to digestion with vertebrate collagenase (MMP1), but not to stromelysin [113].

Depending on the tissue, there is some heterogeneity in the composition of type V and XI molecules. Most tissues contain type V collagen molecules consisting of 2α1(V) and one α2(V) chain, but $[\alpha1(V)]_3$ homotrimers have been isolated from tumor cells [114], and some tissues contain α1(V), α2(V), α3(V) heterotrimers (see Table I). Also type V/XI hybrid molecules containing α1(V) and α2(XI) collagen chains were described in articular cartilage [4, 115], in bone [116], and in vitreous humor [117].

Immunohistochemical identification of type V collagen in tissue sections with antibodies requires demasking of the epitopes with acid or enzymes [118, 119], indicating a dense packing of type V collagen within the type I/V collagen heterofibrils [89].

IV. CARTILAGE COLLAGENS

The backbone of all cartilaginous tissues of the vertebrate body is a heterofibril containing type II collagen as the predominant collagen type, into which type XI collagen is incorporated. The cartilage collagen fibril is decorated with FACIT collagens, mostly type IX collagen, which is covalently linked to type II collagen [7, 120]. The large N-terminal noncollagenous domains of FACIT collagens, which are globular in the case of type IX collagen and cross-shaped in the case of types XII or XIV collagen, reach out of the fibril into the adjacent matrix space and may serve to anchor the collagen fibril in the proteoglycan matrix (see Fig. 2). Young growth cartilage contains about 85–90% type II collagen with 5–10% type XI and 5–10% collagen IX, while adult articular cartilage may have as little as 1% collagen IX and 3% collagen XI [4].

Substantial differences exist in the matrix composition of different cartilaginous tissues. Elastic cartilage contains collagens II, IX, and XI like hyaline cartilage, but elastin in addition. Fibrous cartilage contains a substantial amount of type I collagen besides type II collagen; type I collagen is also found in prechondrogenic tissue, e.g. in the perichondrium, and in tendon insertions [121]. In mammalian articular cartilage, type I collagen is restricted to the articular surface, where also the newly discovered collagen XXII was located [32], while chicken articular cartilage contains up to 30% type I collagen in the upper zone [122]. In osteoarthritic joints type I collagen was found in osteochondrophytes and fibrous repair tissue. There is still a debate on the question whether articular chondrocytes turn on type collagen I synthesis in osteoarthritis. While some studies report on the presence of type I collagen in osteoarthritic cartilage [123, 124], other immunohistological and *in situ* hybridization studies confirm the expression of α2(I) mRNA, but not of type I collagen protein in OA cartilage [125]. In contrast, type III collagen is an integral part of mammalian articular cartilage, where it has been located predominantly in the pericellular environment of chondrocytes [126]. Electron microscopic studies have shown that it is also incorporated in the type II collagen fibril [127].

Recently two new fibril-forming collagens (types XXIV and XXVII) [31, 37], and two new FACIT-like collagens (types XX and XXII) [30, 32] were found to be expressed in bone or cartilage, but their function is not yet known. The cartilage collagen fibrils are interwoven with a microfibrillar mesh consisting of type VI collagen, which is prominent in the chondrons of articular cartilage [9, 11]. Type X collagen, a network-forming collagen, is expressed predominantly in fetal and juvenile hypertrophic growth cartilage. It was also located to the upper zone of articular cartilage in certain joints in human, dog, and mouse cartilage [128–130], and in chondrocyte clusters of osteoarthritic cartilage and osteophytes [131, 132]. It supports endochondral ossification [55, 133, 134] and hematopoietic cell differentiation [135].

As a specific marker for hypertrophic chondrocytes it is widely used to analyze chondrocyte differentiation in skeletal development, but also to describe endochondral ossification in fracture callus and osteophyte formation [132, 136].

A. Type II Collagen

1. Structure and Localization

Type II collagen is found predominantly, but not exclusively, in hyaline cartilage [137, 138], where it accounts for approximately 90% of the total collagen. It is a homotrimeric molecule with the composition $[\alpha1(II)]_3$ with similar size and biochemical features as type I collagen [3, 120], but it contains substantially more hydroxylysine-linked galactosyl-glucosyl disaccharides than type I collagen (ten disaccharides per α1(II) vs two per α1(I) chain). In early embryonic cartilage, type II collagen heterofibrils generally appear as 25–50-nm thin, unbanded fibrils, while in calcified cartilage or after reconstitution *in vitro* type II collagen may form up to 400-nm thick crossbanded fibrils with a 68-nm banding pattern repeat similar to type I collagen. Type II collagen exists in two splice variants; in the IIA splice form which is dominant in mature cartilage, exon 2 coding for a 69 amino acid residue, cysteine-rich domain in the N-terminal propeptide is spliced out. It is retained in the IIA splice variant, a transient embryonic form which was found in prechondrogenic mesenchyme, in perichondrium and vertebrae [139, 140]. Type II collagen is not only the major collagen of hyaline elastic [141] and fibrous cartilage [142, 143], but also represents the major collagen of vitreous humor [137, 144] and the nucleus pulposus of intervertebral disks. Furthermore, type II collagen is synthesized transiently by many embryonic epithelia such as notochord [73], cornea epithelium [72, 73], retina pigment epithelium, cranio facial mesenchyme, and endocardial and mesocardial tissues [71, 145–147].

2. The Role of Type II Collagen in Cartilage Formation and Stability

Much has been learned on the role of type II collagen in cartilage development and function from mutations in the human *COL2A1* gene, causing a rather diverse spectrum of skeletal dysplasias such as achondrogenesis, hypochondroplasia, Stickler syndrome, spondyloepiphyseal dysplasia congenita, and Kniest syndrome [28, 148–150]. As in OI, the more severe forms of chondrodysplasias result from dominant negative mutations affecting triple helix stability such as glycine substitutions, overmodification of the proα1(II) and partial or complete intracellular degradation of type II procollagen molecules containing only one mutated α1(II) chain. For example, in the cartilages of achondrogenesis fetuses with a $Gly^{769}Ser$ mutation [151] or a $Gly^{913}Cys$ mutation causing hypochondrogenesis [152], no type II collagen was found, but instead a matrix containing types I and III collagen. Both mutations are lethal, demonstrating that type I and III collagen cannot replace the function and properties of type II collagen.

Inactivation of the type II collagen gene in mice by homologous recombination had severe consequences on skeletal development, in particular on notochord turnover [69] and vertebral development [153]. Interestingly, it did not affect early embryonic development of the eye, somites or craniofacial tissues, as had been predicted from the expression of type collagen in numerous early embryonic epithelia [71, 146]. Type II collagen-deficient mice die as a result of breathing and weaning inability due to thorax malformation and cleft palate formation, respectively. Long bones are shortened due to impaired endochondral ossification, and vertebral bodies and ribs are abnormal. Similar to achondrogenesis or hypochondrogenesis patients, the cartilaginous tissues contain chondrocyte-like cells, but a matrix consisting of types I and III collagen instead of type II collagen. Although the cartilaginous matrix contains aggrecan, the density of the collagen fibrils was lower and their structure abnormal [153].

Compared to other fibrillar collagens, type II collagen has unique antigenic properties: antibodies raised against chicken type II collagen cross-react with type II collagens from all other species including human, mouse, rat, calf, dog, sheep, and shark [90, 154], indicating highly conserved antigenic epitopes in the type II collagen molecule. Like other matrix components of articular cartilage, which has an immune privilege as a nonvascularized tissue, type II collagen is a major target for autoimmune responses in rheumatoid arthritis [155–157]. In animal models, purified native type II collagen has been shown to induce arthritis in certain strains of mice and rats [158, 159]. The major B-cell epitopes of type II collagen have been identified in the mouse, rat, and human system. They are confomation-dependent and located near the integrin-binding site [160, 161]. Interestingly, the major T-cell epitope in rat and human type II collagen which are MHC restricted includes a galactosyl-glucosyl carbohydrate residue [162].

B. Type XI Collagen

Type XI collagen is a heterotrimer consisting of α1, α2, and α3 (XI) subunits found predominantly in hyaline cartilage and vitreous humor associated with type II collagen. The α3(XI) subunit is identical in its amino acid sequence with α1(II) as it is translated from the *Col2a1* gene, but it differs from α1(II) by a higher degree of hydroxylation and glycosylation [109, 163]. In mature cartilage, half of the α1(XI) molecules are replaced by α1(V) chains [164]. As in type V procollagen, the N-terminal domains of α1(XI) and α2(XI) chains are processed only partially after secretion, leaving stubs of 70–100 kDa in the fibril [163, 165]. Complex alternative splicing occurs within

the aminoterminal noncollagenous domains of α1(XI) and α2(XI) [166–168]. A proline- and arginine-rich subdomain (PARP-domain) of the aminopropetides of α1(XI) and α2(XI) seems to be rather stable and persists in the cartilage matrix [110, 169].

As an integral part of the cartilage collagen fibril, type XI collagen serves as a nucleation site of type II/XI collagen fibril formation and regulates the lateral growth of the fibrils [170, 171]. Within the fibril, type XI collagens are covalently cross-linked to each other through their N-telopeptide to helix interaction sites [7] and to the end of type II collagen molecules. The N-propeptide may stick out of the gap domains in the heterofibril (see Figs 2, 3) [172].

The pivotal role of type XI collagen in control of cartilage collagen fibril assembly became apparent also from the analysis of the gene defect of the cho-mouse mutant, which is affected with an autosomal recessive chondrodysplasia: a point mutation in the *α1(XI)* gene leading to chain determination caused absence of type XI collagen, resulting in a cartilage with irregular thick collagen fibrils, disorganized cartilage growth plate and disturbed chondrocyte differentiation [173]. Similarly, an in-frame deletion in the *α2(XI)* gene caused an autosomal recessive bone dysplasia or autosomal dominant Stickler syndrome [174].

C. Fibril-Associated Collagens with Interrupted Triple Helices (FACIT Collagens)

1. TYPE IX COLLAGEN

First evidence for the presence of additional collagenous proteins in cartilage was obtained in the form of various pepsin-resistant small collagenous fragments [175, 176]. Combined protein chemical and molecular biological efforts led to the elucidation of the complex structure of type IX collagen [177, 178]. It is a heterotrimeric molecule consisting of α1(IX), α2(IX) and α3(IX) chain, with three triple helical segments (COL1–COL3) that are interrupted and flanked by four globular domains NC1-NC4 [179, 180] (Figs 3, 4). Electron microscopical analysis of carefully dissected cartilage collagen fibrils revealed that the highly cationic NC4 domain, the largest domain with 243 amino acid residues and a pI of 9.7, reaches out from the fibril where it presumably interacts with proteoglycans [181]. A "hinge region" in NC3, caused by supernumerary amino acids in the NC3 domain of the α2(IX) chain, allows flexibility in the molecule. It is covalently linked to the surface of cartilage collagen fibrils with the collagenous domains COL1 and COL2 [182, 183]. The α2(IX) chain contains a chondroitin sulfate side chain [184, 185], linked to

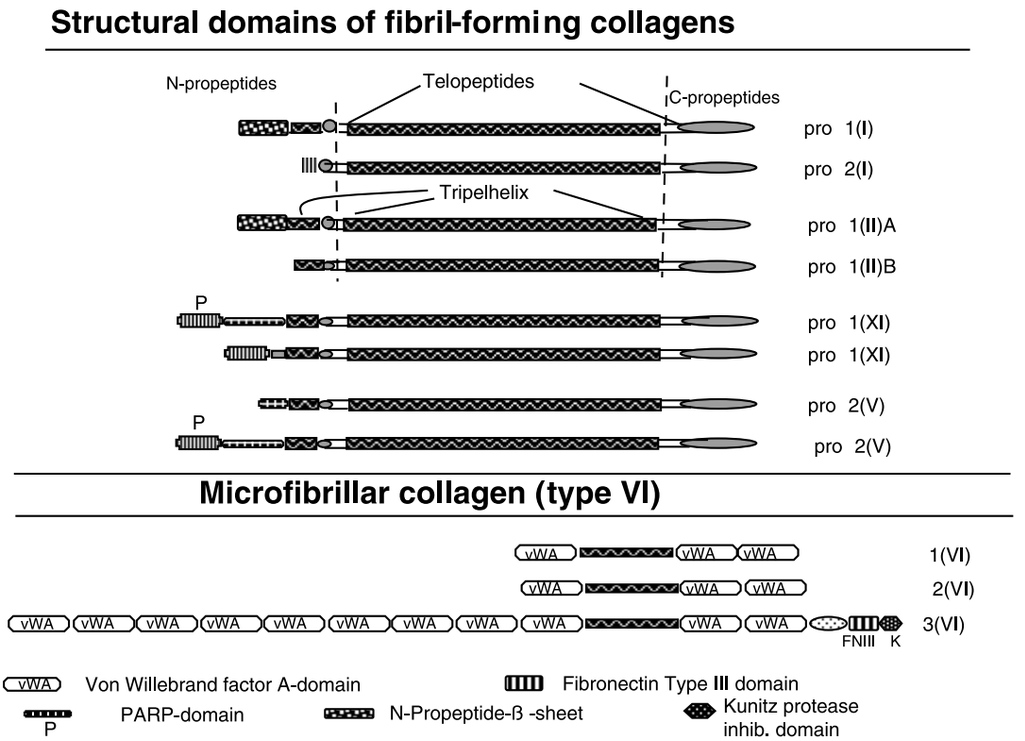

FIGURE 3 Structural domains of fibril-forming collagens and the microfibrillar collagen type VI.

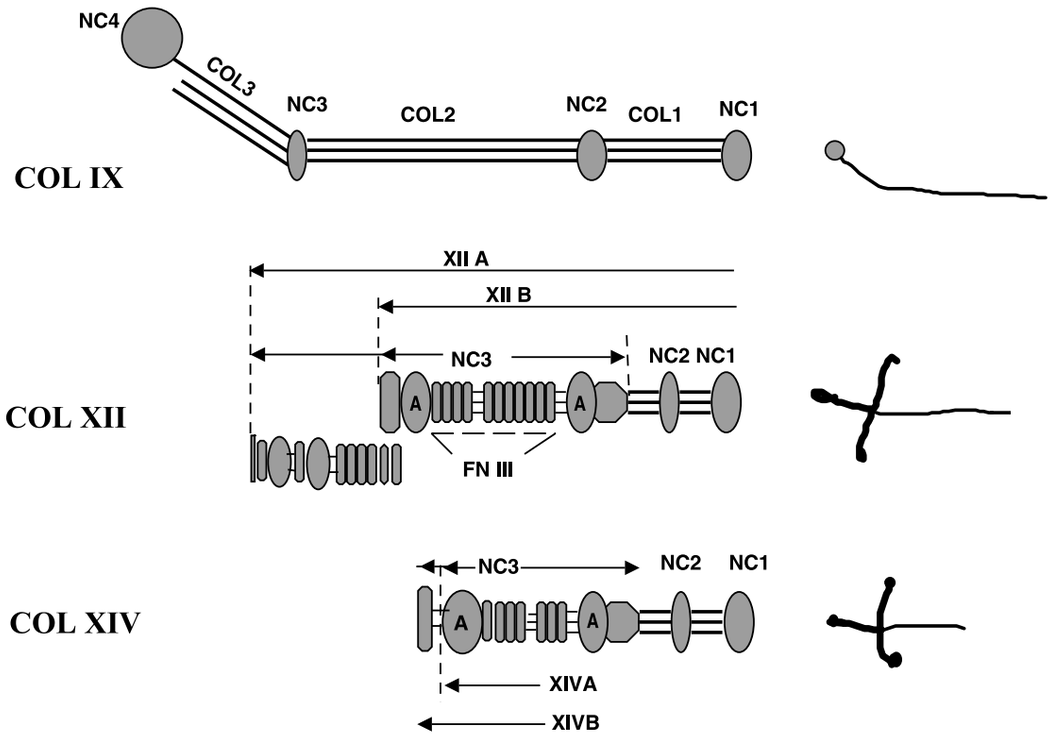

FIGURE 4 Collagenous and noncollagenous domains of the FACIT collagens type IX, XII, and XIV. A = von Willebrand factor A domain.

a serine residue in the NC3 hinge region [186]. This CS chain is considerably longer in vitreous humor than in cartilage [187].

Owing to a second transcription start site between exons 6 and 7 in the *α1(IX)* collagen gene (see below), a shorter form of the α1(IX) collagen lacking the entire NC4 globule is expressed in the cornea and vitreous humor [188, 189]. The importance of type IX collagen for the integrity of cartilage matrix was underlined by the result of inactivation of the *α2(IX)* gene in mice. Animals homozygous for the deficiency in *α2(XI)* showed severe defects in cartilage development and revealed degenerative changes in adult articular cartilage similar to osteoarthritis [190].

2. TYPES XII, XIV, AND XVI COLLAGEN

Type XII collagen is located predominantly in the perichondrium and articular surface, while type XIV collagen is more uniformly distributed throughout articular and tracheal cartilage [191]. Type XII collagen is a homotrimeric molecule with sequence homologies to type IX collagen in the C-terminal NC1 and COL1 domain, but only two collagenous domains which associate with type I or II collagen fibrils [192–194] (Fig. 4). The large, cross-shaped NC3 domain at the amino end reaches out into the perifibrillar space [195]. This domain contains vWFA-domains, TSP and FN type III domains. Type XII collagen exists in two splice variants [196, 197]: the smaller IIB form with an α1(XII) chain of MW 220 kDa is found in skin, periosteum, and perichondrium. The larger XII form with an α-chain of MW 310 kDa was found in an epidermal cell line and contains a chondroitin sulfate chain.

Collagen type XIV has a similar structure, although the cross-shaped NC3 domain is smaller than in Col XII [193, 198]. Type XIV collagen colocalizes with type I collagen in skin, tendon, lung, liver, placenta, and vessel walls by immunofluorescence [199, 200], and to some extent with type XII collagen in skin [201]. However, it does not bind directly to type I collagen, but to the dermatan sulfate side chain of decorin which associates with type I collagen [202, 203].

Collagen XVI is predominatly synthesized by fibroblasts and myoblasts, but also found in the territorial matrix of chondrocytes [204–206]. By immunohistochemistry and immunogold electron microscopy it was shown that collagen XVI is integrated in a discrete population of thin, D-banded collagen fibrils containing type II and XI that are distinct from type IX collagen-containing fibrils [204]. In the papillary dermis, however, collagen XVI is a component of fibrillin-1-containing microfibrils [204].

3. TYPES XIX, XX, XXI, AND XXII COLLAGENS

These recently discovered collagens are phylogenetically and structurally related to FACIT collagens, but whether they are associated with fibrils *in vivo* remains to be shown. Type XIX collagen is restricted to muscle in the embryo, but not in the adult [207, 208]. Collagen XX is most abundant in the corneal epithelium, but by RTPCR it has also been detected in sternal cartilage and tendon [30]. The collagen XXI gene codes for a short FACIT collagen containing a vWFA and a Tsp (thrombospondin)-domain like the other FACIT collagens, but little is known on the protein [209]. Collagen XXII exhibits a restricted localization at tissue junctions such as myotendinous junctions, the hair follicle basement membrane or the articular cartilage–synovial fluid interface [32].

D. Microfibrillar Collagens: Type VI Collagen

Type VI collagen is the major collagenous component of microfibrils in elastic fibers and in a larger variety of tissues including cartilage, skin, blood vessels (intima), cornea, placenta, uterus, ciliary body, iris, and others [2, 210]. The type VI collagen molecule is a highly glycosylated, cysteine-rich heterotrimer consisting of two α-chains of ca. 1000 amino acid residues (α1(VI) and α2(VI)), and the long α3(VI) chain with about 3000 amino acid residues; the short triple helical core accounts only for about 20% of the molecule. It was discovered first in the form of pepsin-resistant "short-chain" collagen with three α-subunits in smooth muscle and placenta [211]. The three α-chains share three noncollagenous domains which are homologous to the von Willebrand factor-A domain [212, 213]; the α3(VI) which exists in multiple splice variants [214–216] contains an additional eight VWF-A-domains at the N-terminus [216]. The C-terminal domain of α3(VI) also contains a Kunitz-type inhibitor motif and is essential for collagen VI assembly and secretion [217]. Interestingly, the α3(VI) subunit is down-regulated by γ-interferon, but the other subunits are not [218]. Type VI collagen interacts with other matrix proteins and proteoglycans, e.g. hyaluronan, heparan sulfate, decorin or NG2-proteoglycan [219], but also with type IV collagen in basement membranes [220].

Examination of type VI collagen in tissues or cell cultures by electron microscopy often reveals beaded filaments with 25-nm beads aligned in 100-nm intervals [221, 222]. Such structures can be assembled *in vitro* from type VI collagen tetramers which connect and overlap at the globular ends when visualized by rotary shadowing [210, 223]. Complexes of matrilin-1 and biglycan or decorin decorate type VI collagen and link it to type II collagen [224]. In the presence of lumican, however, type VI collagen can also assemble into hexagonal networks similar to type X collagen [225]. In tissues like skin or cartilage the type VI collagen forms a highly disulfide cross-linked, branched network, interwoven with fibrillar collagens [9]. Type VI collagen expression is up-regulated already in early stages of chondrocyte differentiation [226]. In mature cartilage it is preferentially located in the pericellular space [8, 9, 11]. It is enhanced in osteoarthritic cartilage [227], but has not been identified yet in calcified bone tissues. In some tissues such as nucleus pulposus and in some tumors type VI collagen filaments may assemble in a parallel fashion to give rise to sheets with the characteristic 100-nm periodicity [222, 228].

The pericellular location of type VI collagen is consistent with its highly adhesive properties for many cell types. In contrast to other fibrillar collagens, several RGD-sequences in the α2-and α3(VI)-chain were found to be recognized by integrin receptors [229].

E. Network Forming Collagens: Type X Collagen

Hypertrophic cartilage in the growth plate of fetal and juvenile long bones, ribs, and vertebrae contains a short-chain collagen, type X collagen [55, 230–232] which is unique to this tissue in the normal organism and only found elsewhere under pathological conditions, e.g. in osteoarthritic articular cartilage and in chondrosarcomas [131, 132, 233–235]. There is, however, recent evidence that type X collagen is also present in small amounts in the menisci and in the surface layer of articular cartilage of certain human, mouse, and dog joints [128–130].

Type X collagen is a homotrimeric collagen with a 130-nm triple helical core (460 amino acids per chain), a large C-terminal globular NC1 domain and a short amino terminal NC2 globule [55, 232]. It is homologous in both sequence and tertiary structure to type VIII collagen, which is produced by endothelial cells [236]. Type VIII collagen assembles into sheets with a hexagonal arrangement in the Descemet's membrane [237], and *in vitro* reconstitution experiments with chicken type X collagen indicate that this collagen is able to form similar structures [238]. The three C-terminal NC1 domains of type X collagen molecule associate with unusually high affinity to a dense bell-shaped trimer [54, 56, 239] which is homologous to the complement factor C1q and adipoQ and TNFα, not only by amino sequence [239] but also by their crystal structure [54]. Mutations in the NC1 domains lead to cartilage growth abnormalities and waddling gait in patients affected with Schmid-type metaphyseal chondrodysplasia (SMCD) [240–245]. *In vitro* studies on the chain assembly