Collagen fibril formation in vitro and in vivo

DOI:
10.1111/iep.12224
10.1111/iep.12224

Document Version
Accepted author manuscript

Fell Muir Review

Collagen fibril formation *in vitro* and *in vivo*

Karl E. Kadler

Wellcome Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT UNITED KINGDOM

e-mail: karl.kadler@manchester.ac.uk

Keywords: collagen, fibril, fibripositor, tendon, electron microscopy, procollagen

Abstract

It is a great honour to be awarded the Fell Muir Prize for 2016 by the British Society of Matrix Biology. As recipient of the prize I am taking the opportunity to write a mini-review on collagen fibrillogenesis, which has been the focus of my research for 33 years. This is the process by which triple helical collagen molecules assemble into centimetre-long fibrils in the extracellular matrix of animals. The fibrils appeared a billion years ago at the dawn of multicellular animal life as the primary scaffold for tissue morphogenesis. The fibrils occur in exquisite three-dimensional architectures that match the physical demands of tissues; for example orthogonal lattices in cornea, basket-weaves in skin and blood vessels, and parallel bundles in tendon, ligament, and nerves. The question of how collagen fibrils are formed was posed at the end of the nineteenth century. Since then we have learned about the structure of DNA and the peptide bond, understood how plants capture the sun's energy, cloned animals, discovered antibiotics, and found ways of editing our genome in the pursuit of new cures for diseases. However, how cells generate tissues from collagen fibrils remains one of the big unsolved mysteries in biology. In this review I will give a personal account of the topic and highlight some of the approaches that my research group are taking to find new insights.

A brief introduction to collagen

There are several excellent reviews on the collagen family and collagen structure (two such examples are (Bella, 2016, Mienaltowski and Birk, 2014)) and therefore only a brief account will be given here.

Collagens are a large family of proteins that have three left-handed polyproline II-like helices wound into a right-handed supercoiled triple helix. The chains have a repeating Gly-X-Y triplet in which glycine is located at every third residue position and X and Y are frequently occupied by the imino acids proline and hydroxyproline (see (Bella*, et al.*, 1994, Brodsky and Persikov, 2005, Brodsky and Ramshaw, 1997) and reviewed by (Bella, 2016)). The first 20-or-so collagens were identified in animal tissues at the protein level and were assigned Roman numerals (reviewed by (Myllyharju and Kivirikko, 2004)). However, with the advent of genome sequencing it became apparent that many more collagens exist. We now know that there are 28 distinct collagens in vertebrates ((Huxley-Jones*, et al.*, 2007) and reviewed by (Kadler*, et al.*, 2007, Mienaltowski and Birk, 2014)), almost 200 in *C. elegans* (reviewed by (Johnstone, 2000)), and further collagens in marine invertebrates (Exposito*, et al.*, 2010, Thurmond and Trotter, 1994, Trotter and Koob, 1989), bacteria (see (Ghosh*, et al.*,

2012) and references therein) and viruses (e.g. see (Legendre*, et al.*, 2011, Rasmussen*, et al.*, 2003)). It has become clear that the triple helix is an important motif that is not restricted to collagens (Brodsky and Shah, 1995) but which occurs in a wide range of proteins including asymmetric acetylcholinesterase (Johnson*, et al.*, 1977), macrophage scavenging receptors (Kodama*, et al.*, 1990), complement component C1q (Reid and Day, 1990), ectodysplasin (Ezer*, et al.*, 1999), and the mannose-binding lectin, collectins, and ficolins in the lectin pathway (Garred*, et al.*, 2016) that are involved in mediating host-pathogen interactions (Berisio and Vitagliano, 2012).

The polypeptide chains in collagens are termed α-chains. Because there are numerous collagen genes and their protein products trimerise in a specific combination to produce a collagen 'type', a nomenclature has evolved to specify a particular α-chain based on the collagen type in which it is found. The nomenclature involves the α symbol followed by an Arabic number followed by a Roman numeral, in brackets (parentheses). The α symbol, Arabic number and Roman numeral are read together to indicate the gene that encodes that particular α-chain. Thus, α1(I) and α2(I) denotes that these chains are found in type I collagen, and are encoded by the genes COL1A1 and COL1A2.

Collagens can be homotrimers and heterotrimers. Moreover, some collagens of the same type can be homotrimeric or heterotrimer (e.g. type I collagen can exist as a homotrimer of three α1(I) chains (i.e. $[\alpha1(I)]_3$) chains or a heterotrimer of two α1(I) chains and a single α2(I) chain (i.e. $[\alpha1(I)]_2$,α2(I)). Furthermore, heterotrimeric collagens can have 3 different α-chains (e.g. α1(IX), α2(IX), α3(IX)) that are encoded by three different genes (i.e. COL9A1, COL9A2 and COL9A3, respectively), and some collagen types contain specific combinations of a family of 6 chains (e.g. $[\alpha1(IV)]_2$,α2(IV) and α3(IV),α4(IV),α5(IV) and α5(IV),α5(IV),α6(IV) (see (Hudson*, et al.*, 2003) for review)). There is chain selection specificity such that of the 45 different collagen α-chains in vertebrates, only 28 different types occur (see Table 1). For fibrillar collagens (Figure 1) the chain selection mechanism resides in the non-collagenous sequences at the C-terminal end of each pro-α-chain (Bourhis*, et al.*, 2012, Lees*, et al.*, 1997). The chain selection mechanism in other collagens is less well understood.

Fibrillar collagens

The 28 collagen types that occur in vertebrates can be classified according to domain structure, function, and supramolecular assembly (for review see (Mienaltowski and Birk, 2014)). The most abundant are the fibrillar collagens that form the basis of the fibrils in

bony, cartilaginous, fibrous, and tubular structures, and will be the focus of the remainder of this review. The fibril-forming collagens are types I, II, III, V, XI, XXIV and XXVII. They have uninterrupted triple helices of ~300 nm in length and have globular domains (propeptides) at each terminus of each α-chain. Types XXIV and XXVII were identified by genome sequencing and were added to this group on the basis of protein domain structure (Koch*, et al.*, 2003) and the presence of type XXVII collagen in thin fibrils (Plumb*, et al.*, 2007).

Collagen fibrils are complex macromolecular assemblies that comprise different fibrillar collagen types (Hansen and Bruckner, 2003). The fibrils are either 'predominately type I collagen' or 'predominately type II collagen'. Predominately type I collagen fibrils occur in bony, tubular, and fibrous tissues whereas cartilaginous tissues contain predominately type II collagen fibrils. Collagen fibrils range in length from a few microns to centimetres (Craig*, et al.*, 1989) and therefore have molecular weights in the tera Dalton range (based on calculations described by (Chapman, 1989)). The fibrils provide attachment sites for a broad range of macromolecules including fibronectin, proteoglycans, and cell surface receptors such as integrins, discoidin domain-containing receptors (DDRs) and mannose receptors (Di Lullo*, et al.*, 2002, Jokinen*, et al.*, 2004, Orgel*, et al.*, 2011, Sweeney*, et al.*, 2008). Furthermore, the fibrils vary in diameter depending on species, tissue, stage of development, (Craig, Birtles, Conway and Parry, 1989, Parry*, et al.*, 1978) and in response to injury and repair (Pingel*, et al.*, 2014). Collagen fibrils are arranged in exquisite three-dimensional architectures *in vivo* including parallel bundles in tendon and ligament, orthogonal lattices in cornea, concentric weaves in bone and blood vessels, and basket-weaves in skin. How the fibrils assemble, how length and diameter are regulated, how molecules attach to fibril surfaces, and how the multi-scale organisation is achieved are questions for which answers are either sketchy or not available. The extreme size and compositional heterogeneity of collagen fibrils mean that they are extremely difficult to study by conventional molecular, genetic and biochemical approaches. Collagen molecules are also hydroxylated at specific prolyl residues (by prolyl hydroxylases), lysyl residues (by lysyl hydroxylases or PLODs, procollagen-lysine 5-dioxygenases) and are non-reducibly cross-linked (by lysyl oxidase (LOX) enzymes), (Eyre*, et al.*, 1984, Gistelinck*, et al.*, 2016), which adds to the difficulty of studying the fibrils.

Collagen fibrils in tendon (Heinemeier*, et al.*, 2013) and cartilage (Heinemeier*, et al.*, 2016) are extremely long lived with estimates exceeding hundreds of years. Therefore the collagen in the fibrils is particularly prone to modification by advanced glycation end products (Thorpe*, et al.*, 2010, Verzijl*, et al.*, 2000). Thus, the two major experimental

approaches used in the study of collagen fibril assembly have been electron microscopy of tissues to describe the organisation of the fibrils *in vivo* (explained below) and reconstitution of fibrils *in vitro* using collagen extracted from tissues (explained below) or recombinant collagens (e.g. (Fertala*, et al.*, 1996)).

Collagen fibril assembly *in vitro*

Studies by Gross (Gross and Kirk, 1958), Wood & Keech (Wood and Keech, 1960), Hodge & Petruska (Hodge, 1989), Silver (Silver and Trelstad, 1980), and Chapman (Bard and Chapman, 1968), to name a few, showed that exposure of animal tissues (typically skin and tendon) to weak acidic solutions (typically acetic acid) or neutral salt buffers yielded a solution of collagen molecules that when neutralised and warmed to ~30°C, produced elongated fibrils that had the same alternating light and dark transmission electron microscope banding appearance as fibrils occurring *in vivo* (Holmes and Chapman, 1979) (Figure 2). The characteristic banding pattern of the fibrils arises from *D*-staggering of triple helical collagen molecules that are 4.4 x *D* in length (where *D* is 67 nm, to a close approximation). The electron-dense stain used at neutral pH penetrates more readily into regions of least protein packing (the 'gaps') between the N- and C-termini of collagen molecules that are aligned head-to-tail along the long axis of the fibril. The fact that fibrils with *D*-periodic banding could be formed *in vitro* from purified collagen showed that all the information required to form a collagen fibril was contained within the amino acid sequence and triple helical structure of the collagen molecule (Hulmes*, et al.*, 1973).

Subsequent studies showed that collagen fibrils from embryonic tendon (which are predominantly type I collagen) exist in two isoforms: unipolar and bipolar (Holmes*, et al.*, 1994) (Figure 3). Unipolar fibrils have all collagen molecules in the fibril oriented in one direction, which gives the fibril a carboxyl and an amino tip. Bipolar fibrils (more precisely, N, N-bipolar fibrils) have two amino terminal ends and a molecular polarity switch region (or transition zone) in which the orientation of collagen molecules switches e.g. from N-to-C to C-to-N (Holmes, Lowe and Chapman, 1994). The switch in orientation occurs over an 8 D-period range in chick tendon collagen fibrils (Holmes, Lowe and Chapman, 1994). Notably, sea cucumbers (*Actinopyga echinites*) lack unipolar fibrils and all their bipolar fibrils have the molecular switch region located precisely mid-way from each fibril tip; also, the switch varies in extent from 14 to 41 *D*-periods in invertebrate fibrils (Trotter*, et al.*, 1998, Trotter*, et al.*, 2000). Earlier studies had shown that collagen fibrils formed by cleavage of procollagen to collagen (explained below) grow from pointed tips (i.e. the pointed ends of fibrils) and the

collagen molecules were oriented in one direction along the long axis of the fibril (Kadler*, et al.*, 1990). Moreover, the C-tip of a unipolar fibril is required for end-to-end fusion of either two unipolar fibrils (to generate a new N, N-bipolar fibril) or to one end of an N, N-bipolar to generate a longer N, N-bipolar fibril (Graham*, et al.*, 2000, Kadler*, et al.*, 2000). Notably, C, C-bipolar collagen fibrils have not been described. Presumably, the structure of C-tips exposes binding sites to promote carboxyl-to-amino fusion of fibril tips.

Two schools of thought developed about how collagen molecules assemble into fibrils: (1) precipitation from a solution of 'bulk' collagen by liquid crystalline ordering of molecules (e.g. see (Martin*, et al.*, 2000)), or (2) 'nucleation and propagation' in which a finite number of collagen molecules form a nucleus that then grows in length and diameter to become the mature fibril (Gross*, et al.*, 1954). This latter mechanism is analogous to the formation of inorganic crystals. The existence of fibrils of different lengths supports the notion that the fibrils grow in size (which supports the nucleation and propagation model) but collagen and procollagen (see below) molecules can form a liquid-like structure when packed in high concentration (which supports the liquid crystalline model). In reality, these two hypotheses might not be mutually exclusive; work by Hulmes and Bruns showed that procollagen molecules can align in zero-D register in secretory vacuoles of fibroblasts (analogous to liquid crystalline packing) (Bruns*, et al.*, 1979, Hulmes*, et al.*, 1983), which might increase the rate of conversion of procollagen to collagen to nucleate fibrils. Therefore it is possible that elements of both assembly mechanisms exist *in vivo*.

A system for generating collagen fibrils *in vitro* starting with procollagen

In 1984 I joined Darwin Prockop's laboratory at UMDNJ, Piscataway, NJ, USA to develop a system of studying collagen fibril formation by cleavage of procollagen with its physiological convertases, the procollagen N- and C-proteinases (Figure 4). Procollagen had previously been shown to be the biosynthetic precursor of collagen (Bellamy and Bornstein, 1971)) and there had been initial success in purifying the N- and C-proteinases that convert procollagen to collagen (Njieha*, et al.*, 1982, Tuderman and Prockop, 1982). With the collaboration of Yoshio Hojima who purified the procollagen N- and C-proteinases from chick tendon (Hojima*, et al.*, 1989, Hojima*, et al.*, 1985), we developed a method of purifying type I procollagen and cleaving it with N-proteinase to generate pCcollagen, and then cleaving the re-purified pCcollagen with the C-proteinase in a bicarbonate buffer. pCcollagen is a cleavage intermediate of procollagen that retains the C-propeptide but lacks the N-propeptide. This system allowed us to study collagen fibril formation in the absence of lysyl

oxidase and crosslink precursors (Eyre*, et al.*, 2008). The presence of crosslink precursors in extracted collagen can affect collagen fibril formation *in vitro* (Herchenhan*, et al.*, 2015). Using this new system of forming fibrils by cleavage of procollagen, we defined the thermodynamic parameters of the assembly process (Kadler*, et al.*, 1987), the temperature dependence of collagen fibril assembly (Kadler*, et al.*, 1988), and showed that the fibrils form as a nucleus that grows at its pointed tips (Kadler, Hojima and Prockop, 1990). These observations indicated that collagen fibrils (in the absence of lysyl oxidase-derived crosslinks) exhibit a critical concentration of assembly, analogous to the self-formation of inorganic crystals. Our ability to purify procollagen from cells paved the way to study how mutations in collagen genes that cause osteogenesis imperfecta affect procollagen structure and fibril assembly. These studies showed that mutations in type I collagen genes can produce procollagen molecules that are 'kinked' (Vogel*, et al.*, 1988), slow the rate of conversion of procollagen to collagen (Lightfoot*, et al.*, 1992), lead to the formation of abnormal collagen fibrils (Kadler*, et al.*, 1991), and impair the ability of collagen fibrils to be mineralised during the formation of bone (Culbert*, et al.*, 1995). These studies led to a better understanding of how mutations in collagen genes can change the structure and processing of collagen molecules and how the resultant collagen fibrils are poorer scaffolds for mineralisation, as occurs in osteogenesis imperfecta *in vivo* (Culbert*, et al.*, 1996). In parallel studies we also showed that the tips are the sites of diameter regulation (Holmes*, et al.*, 1998), that fibrils formed at low C-proteinase/pCollagen ratios bore the closest resemblance to fibrils in vivo (Holmes*, et al.*, 1996), and that the tips of fibrils are paraboloidal in shape (Holmes*, et al.*, 1992).

Collagen fibril formation *in vivo*

Although collagen molecules can spontaneously self assemble into fibrils *in vitro*, additional factors must exist *in vivo* to explain the exquisite three-dimensional supramolecular organisation of fibrils, as well as the regulation of diameter, length and composition, that depend on tissue, stage of development, state of tissue ageing and repair, and which vary in disease. The *in vivo* regulation of collagen fibril formation has been studied for over a century, and although enormous progress has been made, the cellular mechanisms of fibril assembly and organisation *in vivo* remain elusive.

Some of the earliest reports on the existence of collagen fibrils date back to the end of the 19th century and beginning of the 20th century. For example, Mallory described a 'fibrillar substance' produced by connective tissue cells (i.e. fibroblasts) (Mallory, 1903).

Studies of collagen fibrils continued during the 1920s and 1930s during which time several groups attempted to develop methods to observe the assembly of the fibrils *in vivo*. A breakthrough came in 1940 when Mary Stearns published her first observations of fibroblasts secreting and assembling collagen fibres (Stearns, 1940). Her paper is a 'must read' for students of collagen fibril formation; the 46 hand-drawn plates are exquisite. Stearns used the camera lucida to visualise and draw details of cytoplasmic connections between cells, striations within cells, 'vacuoles de secretion', and fibres growing at the cell surface. In so doing, she produced the first evidence that fibroblasts are instrumental in assembling collagen fibrils in tissues. Almost 40 years later, Trelstad and Hayashi used transmission electron microscopy (TEM) to show that collagen fibrils occurred in invaginations of the plasma membrane of embryonic fibroblasts (Trelstad and Hayashi, 1979). A decade later this observation was extended using high-voltage TEM to study collagen fibrillogenesis in cornea as well as embryonic chick tendon (Birk and Trelstad, 1984, Birk and Trelstad, 1985, Birk and Trelstad, 1986, Trelstad and Birk, 1985). In 2006, we used serial section TEM and immunoEM of embryonic tendon to describe a variety of structures at the plasma membrane that contained collagen fibrils, and which we collectively called 'fibripositors' (Canty*, et al.*, 2004). Collectively, these studies demonstrate the exquisite control the cell exerts over the self assembly of collagen fibrils to generate tissues with highly organised collagen matrices.

Fibripositors

In 1989 I returned to the UK as a Wellcome Trust Senior Research Fellow in Basic Biomedical Science, and joined Michael Grant's Department of Medical Biochemistry. During the next 10 years we extended our knowledge of how mutations in collagen genes affect procollagen structure and fibril formation. In collaboration with Peter Byers and Gillian Wallis, these studies focussed on the Ehlers-Danlos syndrome (type VII) that is caused by mutations in COL1A1 and COL1A2 genes that encode the chains of type I procollagen. PhD students Rod Watson, Samantha Lightfoot and Ainsley Culbert, and a postdoc David Holmes, joined my lab and together we showed how mutations in COL1A1 and COL1A2 that cause EDS VII disrupt the structure of procollagen, slow the cleavage of procollagen by N-proteinase, and lead to the 'cauliflower' appearance of collagen fibrils in affected individuals (Culbert, Wallis and Kadler, 1996, Holmes*, et al.*, 1993, Wallis*, et al.*, 1992, Watson*, et al.*, 1998, Watson*, et al.*, 1992). We also studied the function of the CUB domains in bone morphogenetic protein-1, which is a potent procollagen C-proteinase (Canty*, et al.*, 2006,

Garrigue-Antar*, et al.*, 2001, Garrigue-Antar*, et al.*, 2004, Garrigue-Antar*, et al.*, 2002, Hartigan*, et al.*, 2003, Petropoulou*, et al.*, 2005). Here, CUB is an evolutionary conserved protein domain named after its discovery in complement components (C1r/C1s), the sea urchin protein Uegf, and in BMP-1 (for review see (Bork and Beckmann, 1993)).

However, it was during a staff meeting in 2002 that I heard good advice that scientists should change their experimental approach every 10 years. Up until this time, I had used cells as a factory for procollagen production and had overlooked the importance of the cell in fibril assembly. A new postdoc in the lab, Elizabeth Canty, took up the challenge of taking our lab into new, *in vivo*, directions. We were inspired by the work of Hayashi, Trelstad and Birk, and decided to ask questions about how cells regulate fibril assembly and fibril number. With the assistance of David Holmes, Tobias Starborg and Yinhui Lu in the lab, Liz Canty embarked on studying collagen fibril formation in embryonic chick tendon using serial section electron microscopy and 3D reconstruction. Our first paper, in 2004, showed 3D reconstructions from 50 x 100 nm serial sections of embryonic chick tendon, cut perpendicular to the tissue long axis. These were the deepest and most detailed 3D reconstructions at the time and showed finger-like projections of the plasma membrane containing thin collagen fibrils (Figure 5). The 3D reconstructions showed that the projections were part of an invagination of the plasma membrane, and, that the fibril within the invagination and the projection were co-aligned to the long axis of the tendon (Canty, Lu, Meadows, Shaw, Holmes and Kadler, 2004) (Figure 6). We called these structures 'fibripositors' (a portmanteau of 'fibril' and 'depositors'). We also showed that fibripositors are actin-dependent structures (Canty*, et al.*, 2006) that projected into intercellular channels stabilised by cadherin-11 containing junctions (Richardson*, et al.*, 2007).

Serial block face-scanning electron microscopy

The fact that fibripositors are too thin to be seen by light microscopy, and that no marker has been identified that can aid in their visualisation by fluorescence light microscopy, has been a severe hurdle to studies of fibripositor structure, function and formation. Also, the effort and time involved in producing serial sections for electron microscopy is a significant hurdle to further progress; sections can be lost or distorted during processing, and the process requires exceptional skills in ultrathin sectioning and handling. A major breakthrough came with the commercialisation of serial block face-scanning electron microscopy (SBF-SEM) (Denk and Horstmann, 2004). Here, images of a block face are recorded using a scanning electron microscope prior to the removal of a section by an in-

microscope ultramicrotome. The ability to produce serial images without manual sectioning opened up new opportunities to explore fibripositor function. After optimisation of sample preparation and staining, image acquisition and data analysis, Toby Starborg, Nick Kalson and Yinhui Lu showed that we could use SBF-SEM as a semi-high throughput system to examine fibripositor structure and function at the cell-matrix interface (Starborg*, et al.*, 2013) (Figure 7 and Movie). With this new approach we were able to show that fibripositors are the site of fibril assembly in tendon and that non-muscle myosin II is required for fibril transport and formation (Kalson*, et al.*, 2013). We also showed that fibripositor-like structures called keratopodia exist in corneal keratocytes (Young*, et al.*, 2014). SBF-SEM also gave us the opportunity to explore how collagen fibril formation contributes to tendon development. In a *tour de force* of SBF-SEM, Nick Kalson, Yinhui Lu and Susan Taylor outlined a new hypothesis for tendon development in which the number of collagen fibrils is determined by embryonic tendon fibroblasts, and that the growth in lateral size of the tendon is driven by matrix expansion caused by the increase in girth and length of collagen fibrils (Kalson*, et al.*, 2015). SBF-SEM studies have also revealed a new function for membrane type I-matrix metalloproteinase (MT1-MMP or MMP14) in being essential for tendon development (Taylor*, et al.*, 2015). Taylor and colleagues showed that release of collagen fibrils from fibripositors at birth requires MT1-MMP, and that the process does not rely on the cleavage of collagen at the ¾-¼ vertebrate collagenase cleavage site in the molecule.

Negative regulation of collagen fibril formation during intracellular protein trafficking

Canty and co-workers also made the observation that procollagen can be cleaved to collagen prior to secretion by tendon fibroblasts *in vivo*. Evidence that procollagen can be cleaved to collagen within the cell without forming fibrils demonstrates active negative control of the self-assembly properties of collagen fibrillogenesis *in vivo* (Humphries*, et al.*, 2008). These observations are in contrast to what happens in conventional cell culture, in which procollagen is readily purified from the cell culture medium. Presumably the environment of the cell and matrix influence the trafficking of procollagen. A half-way house between *in vivo* and *in vitro* is the use of 3D cell culture systems; Kapacee and colleagues showed that fibroblasts incubated in fibrin gels under linear tension replace the fibrin with collagen fibrils that are aligned parallel to the lines of stress and exhibit features of embryonic fibroblasts *in vivo*, including fibripositors (Bayer*, et al.*, 2010, Kalson*, et al.*, 2011, Kalson*, et al.*, 2010, Kapacee*, et al.*, 2008, Kapacee*, et al.*, 2010). This approach

facilitates studies of the role of cells, in a near-physiological environment with tissue-derived mechanical forces, in assembling collagen fibrils.

Regulators of collagen fibril assembly *in vivo*

The fact that collagen fibrils are comprised of different collagens, that they occur in different numbers and with different diameters and packing densities in different tissues, that the supramolecular organisation of fibrils is different in different tissues, and that collagen molecules provide interaction sites for receptors and a wide range of extracellular matrix molecules, suggests that there are multiple steps in the assembly and organisation of fibrils, and that each step can be error prone. Defective collagen fibrillogenesis can arise from mutations in genes encoding fibrillar collagens (see Table 2), fibril associated collagens with interrupted triple helices that bind to the surfaces of collagens fibrils e.g. type XII and type XIV collagen (Young*, et al.*, 2002), proteoglycans that interact with fibrils e.g. decorin (Danielson*, et al.*, 1997), lumican (Chakravarti*, et al.*, 1998) fibromodulin (Hedlund*, et al.*, 1994, Svensson*, et al.*, 1999), osteoglycin (Tasheva*, et al.*, 2002), keratocan (Liu*, et al.*, 2003), and biglycan (Heegaard*, et al.*, 2007) (for review see (Kalamajski and Oldberg, 2010)), enzymes required for posttranslational modification of collagen α-chains e.g. prolyl 4-hydroxylase (Mussini*, et al.*, 1967), lysyl hydroxylases (Takaluoma*, et al.*, 2007), lysyl oxidases (Maki*, et al.*, 2002), proteins involved in transporting collagens through the secretory pathway e.g. HSP47 (Satoh*, et al.*, 1996), sedlin (Venditti*, et al.*, 2012), and TANGO1 (Saito*, et al.*, 2009, Wilson*, et al.*, 2011), and proteinases involved in collagen turnover e.g. MMP14 (Taylor, Yeung, Kalson, Lu, Zigrino, Starborg, Warwood, Holmes, Canty-Laird, Mauch and Kadler, 2015). Loss of the collagen network in cartilage occurs in end stage osteoarthritis (Ehrlich*, et al.*, 1977). Conversely ectopic or excessive accumulation of collagen occurs in fibrosis, which can be stimulated by TGF-β (Roberts*, et al.*, 1986), and can affect any organ often resulting in death. Thus, collagen fibrillogenesis is a precisely regulated process in which the mechanisms that maintain the appropriate number, size, and organisation of collagen fibrils in adult tissues appear to be sensitive to a wide range of genetic mutations and environmental stimuli.

A personal perspective on some of the most important unanswered questions in the field of collagen fibril homeostasis

We do not have clear line of sight of how the three-dimensional organisation of collagen fibrils is established in tissues. Collagen fibrils first appear part way though vertebrate

embryonic development when the mass of matrix begins to exceed the mass of cells. At this pivotal stage of development, the patterning of tissue progenitor cells has, to a close approximation, been established and might be expected to dictate the patterning of the matrix. Perhaps novel insights into organogenesis will come from a better understanding of the interplay between cell positioning, cell-cell communication, cell-matrix interactions, cell polarity, the role of the secretory pathway in directing matrix assembly, and mechanical forces.

A further exciting area of research is matrix homeostasis; it will be fascinating to learn how changes in this process lead to diseases such as osteoarthritis, tendinopathies, fibrosis and cell migration through the matrix. The realisation that the bulk of the collagen in tendon and cartilage is synthesised during adolescence and remains unchanged during the lifetime of a person raises intriguing questions about how the collagen network is maintained during life despite countless cycles of mechanical loading. Advances in genome editing and super-resolution light microscopy are all likely to be brought to bear on this question. These approaches are expected to lead to a better understanding of how matrix homeostasis goes wrong in diseases such as fibrosis, where ectopic and excessive deposition of collagen fibrils can cause death. Recent discoveries show that matrix-rich tissues are peripheral circadian clock tissues and that defects in the rhythm in these tissues lead to pathologies such as calcific tendinopathy (Yeung*, et al.*, 2014), osteoarthritis (Dudek*, et al.*, 2016, Guo*, et al.*, 2015) and intervertebral disc disease (Dudek*, et al.*, 2016). Thus, the mechanical environment of the cell, the role of the matrix in modulating cell behaviour, and peripheral circadian clocks are all likely to contribute to matrix homeostasis.

Acknowledgements

I am indebted to The Wellcome Trust for continuous support of my research for over 30 years. Numerous students, postdoctoral fellows and technical staff have made valuable contributions to this research. However, a few people need a special mention: Laure Garrigue-Antar, Samantha Lightfoot and Rod Watson performed the studies on osteogenesis imperfecta, the Ehlers-Danlos syndrome and bone morphogenetic protein-1 that established my laboratory at the University of Manchester. In recent years, my gratitude goes out to Nick Kalson, Zoher Kapacee, Elizabeth Laird (nee Canty), Yinhui Lu, Susan Taylor (nee Richardson), Toby Starborg, and Chloé Yeung who led the work on 3D electron microscopy, fibripositors, and tendon-like constructs. Finally, very special thanks to David Holmes, who I have known since my PhD days, and who joined my group in the 1990s. David is an

390 enormously talented electron microscopist who has made outstanding contributions to our
391 research.
392

Table 1. Collagen types and their chain compositions

Type	Gene	α-chain	Molecules
I	COL1A1	α1(I)	$[\alpha1(I)]_3$
	COL1A2	α2(I)	$[\alpha1(I)]_2\alpha2(I)$
II	COL2A1	α1(II)	$[\alpha1(II)]_3$
III	COL3A1	α1(III)	$[\alpha1(III)]_3$
IV	COL4A1	α1(IV)	$[\alpha1(IV)]_2\alpha2(IV)$ α3(IV), α4(IV), α5(IV) α5(IV), α5(IV), α6(IV)
	COL4A2	α2(IV)	
	COL4A3	α3(IV)	
	COL4A4	α4(IV)	
	COL4A5	α5(IV)	
	COL4A6	α6(IV)	
V	COL5A1	α1(V)	$[\alpha1(V)]_2\alpha2(V)$ $\alpha1(V), \alpha2(V), \alpha3(V)]_3$ $[\alpha3(V)]_3$
	COL5A2	α2(V)	
	COL5A3	α3(V)	
VI	COL6A1	α1(VI)	α1(VI), α2(VI) and any of α3(VI), α4(VI), α5(VI), and α6(VI) (see (Maass, *et al.*, 2016))
	COL6A1	α2(VI)	
	COL6A1	α3(VI)	
	COL6A1	α4(VI)	
	COL6A1	α5(VI)	
	COL6A1	α6(VI)	
VII	COL7A1	α1(VII)	$[\alpha1(VII)]_3$
VIII	COL8A1	α1(VIII)	$[\alpha1(VIII)]_3$
	COL8A2	α2(VIII)	$[\alpha2(VIII)]_3$
IX	COL9A1	α1(IX)	α1(IX), α2(IX), α3(IX)
	COL9A2	α2(IX)	
	COL9A3	α3(IX)	
X	COL10A1	α1(X)	$[\alpha1(X)]_3$
XI	COL11A1	α1(XI)	$[\alpha1(XI)]_2\alpha2(XI)$; α1(XI), α2(XI), α3(XI)* $[\alpha2(XI)]_3$
	COL11A2	α2(XI)	
XII	COL12A1	α1(XII)	$[\alpha1(XII)]_3$
XIII	COL13A1	α1(XIII)	$[\alpha1(XIII)]_3$
XIV	COL14A1	α1(XIV)	$[\alpha1(XIV)]_3$
XV	COL15A1	α1(XV)	$[\alpha1(V)]_3$
XVI	COL16A1	α1(XVI)	
XVII	COL17A1	α1(XVII)	
XVIII	COL18A1	α1(XVIII)	
XIX	COL19A1	α1(XIX)	
XX	COL20A1	α1(XX)	
XXI	COL21A1	α1(XXI)	
XXII	COL22A1	α1(XXII)	
XXIII	COL23A1	α1(XXIII)	
XXIV	COL24A1	α1(XXIV)	
XXV	COL25A1	α1(XXV)	
XXVI	COL26A1	α1(XXVI)	
XXVII	COL27A1	α1(XXVII)	$[\alpha1(XXVII)]_3$
XXVIII	COL28A1	α1(XXVIII)	$[\alpha1(XXVIII)]_3$

* the α3(XI chain is encoded by the COL2A1 gene

Table 2. Diseases caused by mutations in genes encoding fibrillar collagens

Collagen type	Gene	OMIM	Disease	Mouse models
I	COL1A1	120150	Osteogenesis imperfecta (OI);	Mov13 (Bonadio, *et al.*,

			Ehlers Danlos syndrome type VII	1990); Col1a1(Jrt/+) OI/EDS mouse (Chen*, et al.*, 2014)
	COL1A2	120160	Osteogenesis imperfecta (OI); Ehlers-Danlos syndrome type VII	OIM (Chipman*, et al.*, 1993); Col1a2(+/G610C) OI (Amish) mouse (Daley*, et al.*, 2010)
II	COL2A1	120140	Stickler syndrome; Achondrogenesis; Familial avascular necrosis of the femoral head; Legg-Calves-Perthes disease Kniest dysplasia; Spondyloepiphyseal dysplasia congenitial (SEDC); Czech dysplasia; Myopia 2; Marshall syndrome; Epiphyseal dysplasia; Platyspondylic lethal skeletal dysplasia	(Donahue*, et al.*, 2003, Gaiser*, et al.*, 2002, Garofalo*, et al.*, 1991, Li*, et al.*, 1995, Vandenberg*, et al.*, 1991);
III	COL3A1	120180	Ehlers Danlos syndrome type IV; Intracranial berry aneurysm	(Liu*, et al.*, 1997); Tsk2 mouse (Long*, et al.*, 2015)
V	COL5A1	120215	Nail patella syndrome; Ehlers Danlos syndrome classic type	(Wenstrup*, et al.*, 2004)
	COL5A2	120190	Ehlers Danlos syndrome type I or type II	(Andrikopoulos*, et al.*, 1995)
	COL5A3	120216		(Huang*, et al.*, 2011)
XI	COL11A1	120280	Stickler syndrome; Otospondylomegaepiphyseal dysplasia (OSMED); Marshall syndrome	Cho/cho mouse (Li*, et al.*, 1995)
	COL11A2	120290	Stickler syndrome; Otospondylomegaepiphyseal dysplasia	(McGuirt*, et al.*, 1999); (Li*, et al.*, 2001)
XXIV	COL24A1	610025		
XXVII	COL27A1	608461	Steel syndrome (Gonzaga-Jauregui*, et al.*, 2015)	(Plumb*, et al.*, 2011)

398

399

Figure legends

Figure 1: Schematic diagram of the chain composition of the fibril-forming collagens

Figure 2: Transmission electron microscopy of individual collagen fibrils

A. Single collagen fibril from 18-day chick embryonic metatarsal tendon. The fibril is negatively stained with 2% uranyl acetate to show the characteristic light and dark banding pattern.
B. Schematic representation of the axial arrangement of collagen molecules in a collagen fibril. Each collagen molecule is represented with three coiled chains with amino and carboxy termini indicated. Each molecule is 4.4 x D in length, where D ~ 67 nm. The D-stagger of molecules that are 4.4D long leads to the formation of a gap zone in the axial structure.
C. The characteristic negative staining pattern of collagen fibrils, as shown by 1% sodium phosphotungstate staining at neutral pH.

Figure 3: Unipolar and bipolar collagen fibrils

A. Negatively-stained unipolar collagen fibril isolated from embryonic chick metatarsal tendon. Analysis of the staining pattern shows that the collagen molecules are oriented with their amino terminal to the right hand side (as shown) and the carboxy termini to the left.
B. Negatively-stained N, N-bipolar collagen fibril from embryonic chick tendon showing the molecular polarity switch region (box).
C. Enlargement of the box in B.

Figure 4: Schematic representation of collagen fibril formation by cleavage of procollagen

Sequential cleavage of the N-propeptides (by procollagen N-proteinase, which are ADAM 2, 3, 14) and the C-propeptides (by procollagen C-proteinase, which are the BMP-1/Tolloid family) of procollagen generates collagen that self-assembles into unipolar collagen fibrils (Kadler, Hojima and Prockop, 1987).

Figure 5. Transmission electron microscopy of embryonic tendon

Embryonic tendon contains bundles of collagen fibrils between adjacent fibroblasts. The image shows profiles of fibripositors.

Figure 6: A fibripositor at the plasma membrane of an embryonic fibroblast

Transmission electron microscope image of a collagen fibril contained within a fibripositor at the surface of an embryonic mouse tail-tendon fibroblast.

Figure 7: Serial block face-scanning electron microscopy for studies of the cell-matrix interface

Three images from the downloadable Movie generated by serial block face-scanning electron microscopy. The coloured circles show fibripositors. Numbers refer to the image sequence.

Movie: Step-through movie of consecutive images of embryonic mouse tendon generated by serial block face-scanning electron microscopy

E17.5 mouse-tail tendon was prepared for serial block face-scanning electron microscopy as described previously (Starborg, Kalson, Lu, Mironov, Cootes, Holmes and Kadler, 2013). Images were recorded prior to 100 nm-thick sections being removed sequentially from the block face. The movie shows 60 consecutive images covering a z-depth of 6 µm. Fibripositors are highlighted with coloured circles.

References

Andrikopoulos K., Liu X., Keene D.R., Jaenisch R. & Ramirez F. (1995) Targeted mutation in the col5a2 gene reveals a regulatory role for type V collagen during matrix assembly. Nat Genet 9, 31-36.

Bard J.B. & Chapman J.A. (1968) Polymorphism in collagen fibrils precipitated at low pH. Nature 219, 1279-1280.

Bayer M.L., Yeung C.Y., Kadler K.E., Qvortrup K., Baar K., Svensson R.B., Magnusson S.P., Krogsgaard M., Koch M. & Kjaer M. (2010) The initiation of embryonic-like collagen fibrillogenesis by adult human tendon fibroblasts when cultured under tension. Biomaterials 31, 4889-4897.

Bella J. (2016) Collagen structure: new tricks from a very old dog. Biochem J 473, 1001-1025.

Bella J., Eaton M., Brodsky B. & Berman H.M. (1994) Crystal and molecular structure of a collagen-like peptide at 1.9 A resolution. Science 266, 75-81.

Bellamy G. & Bornstein P. (1971) Evidence for procollagen, a biosynthetic precursors of collagen. Proc Natl Acad Sci U S A 68, 1138-1142.

Berisio R. & Vitagliano L. (2012) Polyproline and triple helix motifs in host-pathogen recognition. Curr Protein Pept Sci 13, 855-865.

Birk D.E. & Trelstad R.L. (1984) Extracellular compartments in matrix morphogenesis: collagen fibril, bundle, and lamellar formation by corneal fibroblasts. The Journal of cell biology 99, 2024-2033.

Birk D.E. & Trelstad R.L. (1985) Fibroblasts create compartments in the extracellular space where collagen polymerizes into fibrils and fibrils associate into bundles. Ann N Y Acad Sci 460, 258-266.

Birk D.E. & Trelstad R.L. (1986) Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation. Journal of Cell Biology 103, 231-240.

Bonadio J., Saunders T.L., Tsai E., Goldstein S.A., Morris-Wiman J., Brinkley L., Dolan D.F., Altschuler R.A., Hawkins J.E., Jr., Bateman J.F. & et al. (1990) Transgenic mouse model of the mild dominant form of osteogenesis imperfecta. Proc Natl Acad Sci U S A 87, 7145-7149.

490 Bork P. & Beckmann G. (1993) The CUB domain. A widespread module in developmentally
491 regulated proteins. J Mol Biol 231, 539-545.

492 Bourhis J.M., Mariano N., Zhao Y., Harlos K., Exposito J.Y., Jones E.Y., Moali C., Aghajari
493 N. & Hulmes D.J. (2012) Structural basis of fibrillar collagen trimerization and related
494 genetic disorders. Nature structural & molecular biology 19, 1031-1036.

495 Brodsky B. & Persikov A.V. (2005) Molecular structure of the collagen triple helix.
496 Advances in protein chemistry 70, 301-339.

497 Brodsky B. & Ramshaw J.A. (1997) The collagen triple-helix structure. Matrix Biol 15,
498 545-554.

499 Brodsky B. & Shah N.K. (1995) Protein motifs. 8. The triple-helix motif in proteins. Faseb
500 J 9, 1537-1546.

501 Bruns R.R., Hulmes D.J., Therrien S.F. & Gross J. (1979) Procollagen segment-long-
502 spacing crystallites: their role in collagen fibrillogenesis. Proceedings of the National
503 Academy of Sciences of the United States of America 76, 313-317.

504 Canty E.G., Garrigue-Antar L. & Kadler K.E. (2006) A complete domain structure of
505 Drosophila tolloid is required for cleavage of short gastrulation. Journal of Biological
506 Chemistry 281, 13258-13267.

507 Canty E.G., Lu Y., Meadows R.S., Shaw M.K., Holmes D.F. & Kadler K.E. (2004)
508 Coalignment of plasma membrane channels and protrusions (fibripositors) specifies the
509 parallelism of tendon. Journal of Cell Biology 165, 553-563.

510 Canty E.G., Starborg T., Lu Y., Humphries S.M., Holmes D.F., Meadows R.S., Huffman A.,
511 O'Toole E.T. & Kadler K.E. (2006) Actin filaments are required for fibripositor-mediated
512 collagen fibril alignment in tendon. J Biol Chem 281, 38592-38598.

513 Chakravarti S., Magnuson T., Lass J.H., Jepsen K.J., LaMantia C. & Carroll H. (1998)
514 Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the
515 absence of lumican. J Cell Biol 141, 1277-1286.

516 Chapman J.A. (1989) The regulation of size and form in the assembly of collagen fibrils in
517 vivo. Biopolymers 28, 1367-1382.

518 Chen F., Guo R., Itoh S., Moreno L., Rosenthal E., Zappitelli T., Zirngibl R.A., Flenniken A.,
519 Cole W., Grynpas M., Osborne L.R., Vogel W., Adamson L., Rossant J. & Aubin J.E. (2014)
520 First mouse model for combined osteogenesis imperfecta and Ehlers-Danlos syndrome. J
521 Bone Miner Res 29, 1412-1423.

522 Chipman S.D., Sweet H.O., McBride D.J., Jr., Davisson M.T., Marks S.C., Jr., Shuldiner
523 A.R., Wenstrup R.J., Rowe D.W. & Shapiro J.R. (1993) Defective pro alpha 2(I) collagen

524 synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. Proc
525 Natl Acad Sci U S A 90, 1701-1705.

526 Craig A.S., Birtles M.J., Conway J.F. & Parry D.A. (1989) An estimate of the mean length
527 of collagen fibrils in rat tail-tendon as a function of age. Connective Tissue Research 19,
528 51-62.

529 Culbert A.A., Lowe M.P., Atkinson M., Byers P.H., Wallis G.A. & Kadler K.E. (1995)
530 Substitutions of aspartic acid for glycine-220 and of arginine for glycine-664 in the triple
531 helix of the pro alpha 1(I) chain of type I procollagen produce lethal osteogenesis
532 imperfecta and disrupt the ability of collagen fibrils to incorporate crystalline
533 hydroxyapatite. The Biochemical journal 311 (Pt 3), 815-820.

534 Culbert A.A., Wallis G.A. & Kadler K.E. (1996) Tracing the pathway between mutation and
535 phenotype in osteogenesis imperfecta: isolation of mineralization-specific genes. Am J Med
536 Genet 63, 167-174.

537 Daley E., Streeten E.A., Sorkin J.D., Kuznetsova N., Shapses S.A., Carleton S.M.,
538 Shuldiner A.R., Marini J.C., Phillips C.L., Goldstein S.A., Leikin S. & McBride D.J., Jr.
539 (2010) Variable bone fragility associated with an Amish COL1A2 variant and a knock-in
540 mouse model. J Bone Miner Res 25, 247-261.

541 Danielson K.G., Baribault H., Holmes D.F., Graham H., Kadler K.E. & Iozzo R.V. (1997)
542 Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin
543 fragility. Journal of Cell Biology 136, 729-743.

544 Denk W. & Horstmann H. (2004) Serial block-face scanning electron microscopy to
545 reconstruct three-dimensional tissue nanostructure. PLoS Biol 2, e329.

546 Di Lullo G.A., Sweeney S.M., Korkko J., Ala-Kokko L. & San Antonio J.D. (2002) Mapping
547 the ligand-binding sites and disease-associated mutations on the most abundant protein in
548 the human, type I collagen. The Journal of Biological Chemistry 277, 4223-4231.

549 Donahue L.R., Chang B., Mohan S., Miyakoshi N., Wergedal J.E., Baylink D.J., Hawes N.L.,
550 Rosen C.J., Ward-Bailey P., Zheng Q.Y., Bronson R.T., Johnson K.R. & Davisson M.T.
551 (2003) A missense mutation in the mouse Col2a1 gene causes spondyloepiphyseal
552 dysplasia congenita, hearing loss, and retinoschisis. J Bone Miner Res 18, 1612-1621.

553 Dudek M., Gossan N., Yang N., Im H.J., Ruckshanthi J.P., Yoshitane H., Li X., Jin D., Wang
554 P., Boudiffa M., Bellantuono I., Fukada Y., Boot-Handford R.P. & Meng Q.J. (2016) The
555 chondrocyte clock gene Bmal1 controls cartilage homeostasis and integrity. J Clin Invest
556 126, 365-376.

557 Dudek M., Yang N., Ruckshanthi J.P., Williams J., Borysiewicz E., Wang P., Adamson A., Li
558 J., Bateman J.F., White M.R., Boot-Handford R.P., Hoyland J.A. & Meng Q.J. (2016) The

559 intervertebral disc contains intrinsic circadian clocks that are regulated by age and
560 cytokines and linked to degeneration. Ann Rheum Dis.

561 Ehrlich M.G., Mankin H.J., Jones H., Wright R., Crispen C. & Vigliani G. (1977)
562 Collagenase and collagenase inhibitors in osteoarthritic and normal cartilage. J Clin Invest
563 59, 226-233.

564 Exposito J.Y., Valcourt U., Cluzel C. & Lethias C. (2010) The fibrillar collagen family.
565 International journal of molecular sciences 11, 407-426.

566 Eyre D.R., Paz M.A. & Gallop P.M. (1984) Cross-linking in collagen and elastin. Annu Rev
567 Biochem 53, 717-748.

568 Eyre D.R., Weis M.A. & Wu J.J. (2008) Advances in collagen cross-link analysis. Methods
569 45, 65-74.

570 Ezer S., Bayes M., Elomaa O., Schlessinger D. & Kere J. (1999) Ectodysplasin is a
571 collagenous trimeric type II membrane protein with a tumor necrosis factor-like domain
572 and co-localizes with cytoskeletal structures at lateral and apical surfaces of cells. Hum Mol
573 Genet 8, 2079-2086.

574 Fertala A., Holmes D.F., Kadler K.E., Sieron A.L. & Prockop D.J. (1996) Assembly in vitro
575 of thin and thick fibrils of collagen II from recombinant procollagen II. The monomers in
576 the tips of thick fibrils have the opposite orientation from monomers in the growing tips of
577 collagen I fibrils. The Journal of biological chemistry 271, 14864-14869.

578 Gaiser K.G., Maddox B.K., Bann J.G., Boswell B.A., Keene D.R., Garofalo S. & Horton W.A.
579 (2002) Y-position collagen II mutation disrupts cartilage formation and skeletal
580 development in a transgenic mouse model of spondyloepiphyseal dysplasia. J Bone Miner
581 Res 17, 39-47.

582 Garofalo S., Vuorio E., Metsaranta M., Rosati R., Toman D., Vaughan J., Lozano G., Mayne
583 R., Ellard J., Horton W. & et al. (1991) Reduced amounts of cartilage collagen fibrils and
584 growth plate anomalies in transgenic mice harboring a glycine-to-cysteine mutation in the
585 mouse type II procollagen alpha 1-chain gene. Proc Natl Acad Sci U S A 88, 9648-9652.

586 Garred P., Genster N., Pilely K., Bayarri-Olmos R., Rosbjerg A., Ma Y.J. & Skjoedt M.O.
587 (2016) A journey through the lectin pathway of complement-MBL and beyond. Immunol
588 Rev 274, 74-97.

589 Garrigue-Antar L., Barker C. & Kadler K.E. (2001) Identification of amino acid residues in
590 bone morphogenetic protein-1 important for procollagen C-proteinase activity. The Journal
591 of biological chemistry 276, 26237-26242.

592 Garrigue-Antar L., Francois V. & Kadler K.E. (2004) Deletion of epidermal growth factor-
593 like domains converts mammalian tolloid into a chordinase and effective procollagen C-
594 proteinase. The Journal of biological chemistry 279, 49835-49841.

595 Garrigue-Antar L., Hartigan N. & Kadler K.E. (2002) Post-translational modification of
596 bone morphogenetic protein-1 is required for secretion and stability of the protein. The
597 Journal of biological chemistry 277, 43327-43334.

598 Ghosh N., McKillop T.J., Jowitt T.A., Howard M., Davies H., Holmes D.F., Roberts I.S. &
599 Bella J. (2012) Collagen-like proteins in pathogenic E. coli strains. PLoS One 7, e37872.

600 Gistelinck C., Witten P.E., Huysseune A., Symoens S., Malfait F., Larionova D., Simoens P.,
601 Dierick M., Van Hoorebeke L., De Paepe A., Kwon R.Y., Weis M., Eyre D.R., Willaert A. &
602 Coucke P.J. (2016) Loss of Type I Collagen Telopeptide Lysyl Hydroxylation Causes
603 Musculoskeletal Abnormalities in a Zebrafish Model of Bruck Syndrome. J Bone Miner Res.

604 Gonzaga-Jauregui C., Gamble C.N., Yuan B., Penney S., Jhangiani S., Muzny D.M., Gibbs
605 R.A., Lupski J.R. & Hecht J.T. (2015) Mutations in COL27A1 cause Steel syndrome and
606 suggest a founder mutation effect in the Puerto Rican population. Eur J Hum Genet 23,
607 342-346.

608 Graham H.K., Holmes D.F., Watson R.B. & Kadler K.E. (2000) Identification of collagen
609 fibril fusion during vertebrate tendon morphogenesis. The process relies on unipolar fibrils
610 and is regulated by collagen-proteoglycan interaction. Journal of Molecular Biology 295,
611 891-902.

612 Gross J., Highberger J.H. & Schmitt F.O. (1954) Collagen Structures Considered as States
613 of Aggregation of a Kinetic Unit. The Tropocollagen Particle. Proc Natl Acad Sci U S A 40,
614 679-688.

615 Gross J. & Kirk D. (1958) The heat precipitation of collagen from neutral salt solutions:
616 some rate-regulating factors. J Biol Chem 233, 355-360.

617 Guo B., Yang N., Borysiewicz E., Dudek M., Williams J.L., Li J., Maywood E.S., Adamson A.,
618 Hastings M.H., Bateman J.F., White M.R., Boot-Handford R.P. & Meng Q.J. (2015)
619 Catabolic cytokines disrupt the circadian clock and the expression of clock-controlled genes
620 in cartilage via an NFsmall ka, CyrillicB-dependent pathway. Osteoarthritis Cartilage 23,
621 1981-1988.

622 Hansen U. & Bruckner P. (2003) Macromolecular specificity of collagen fibrillogenesis:
623 fibrils of collagens I and XI contain a heterotypic alloyed core and a collagen I sheath. The
624 Journal of Biological Chemistry 278, 37352-37359.

625 Hartigan N., Garrigue-Antar L. & Kadler K.E. (2003) Bone morphogenetic protein-1 (BMP-
626 1). Identification of the minimal domain structure for procollagen C-proteinase activity.
627 The Journal of biological chemistry 278, 18045-18049.

628 Hedlund H., Mengarelli-Widholm S., Heinegard D., Reinholt F.P. & Svensson O. (1994)
629 Fibromodulin distribution and association with collagen. Matrix Biol 14, 227-232.

630 Heegaard A.M., Corsi A., Danielsen C.C., Nielsen K.L., Jorgensen H.L., Riminucci M., Young
631 M.F. & Bianco P. (2007) Biglycan deficiency causes spontaneous aortic dissection and
632 rupture in mice. Circulation 115, 2731-2738.

633 Heinemeier K.M., Schjerling P., Heinemeier J., Magnusson S.P. & Kjaer M. (2013) Lack of
634 tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. Faseb J
635 27, 2074-2079.

636 Heinemeier K.M., Schjerling P., Heinemeier J., Moller M.B., Krogsgaard M.R., Grum-
637 Schwensen T., Petersen M.M. & Kjaer M. (2016) Radiocarbon dating reveals minimal
638 collagen turnover in both healthy and osteoarthritic human cartilage. Sci Transl Med 8,
639 346ra390.

640 Herchenhan A., Uhlenbrock F., Eliasson P., Weis M., Eyre D., Kadler K.E., Magnusson S.P.
641 & Kjaer M. (2015) Lysyl Oxidase Activity Is Required for Ordered Collagen Fibrillogenesis
642 by Tendon Cells. J Biol Chem 290, 16440-16450.

643 Hodge A.J. (1989) Molecular models illustrating the possible distributions of 'holes' in
644 simple systematically staggered arrays of type I collagen molecules in native-type fibrils.
645 Connect Tissue Res 21, 137-147.

646 Hojima Y., McKenzie J.A., van der Rest M. & Prockop D.J. (1989) Type I procollagen N-
647 proteinase from chick embryo tendons. Purification of a new 500-kDa form of the enzyme
648 and identification of the catalytically active polypeptides. The Journal of biological
649 chemistry 264, 11336-11345.

650 Hojima Y., van der Rest M. & Prockop D.J. (1985) Type I procollagen carboxyl-terminal
651 proteinase from chick embryo tendons. Purification and characterization. The Journal of
652 biological chemistry 260, 15996-16003.

653 Holmes D.F. & Chapman J.A. (1979) Axial mass distributions of collagen fibrils grown in
654 vitro: results for the end regions of early fibrils. Biochem Biophys Res Commun 87, 993-
655 999.

656 Holmes D.F., Chapman J.A., Prockop D.J. & Kadler K.E. (1992) Growing tips of type I
657 collagen fibrils formed in vitro are near-paraboloidal in shape, implying a reciprocal
658 relationship between accretion and diameter. Proceedings of the National Academy of
659 Sciences of the United States of America 89, 9855-9859.

660 Holmes D.F., Graham H.K. & Kadler K.E. (1998) Collagen fibrils forming in developing
661 tendon show an early and abrupt limitation in diameter at the growing tips. Journal of
662 Molecular Biology 283, 1049-1058.

663 Holmes D.F., Lowe M.P. & Chapman J.A. (1994) Vertebrate (chick) collagen fibrils formed
664 in vivo can exhibit a reversal in molecular polarity. Journal of Molecular Biology 235, 80-
665 83.

666 Holmes D.F., Watson R.B., Chapman J.A. & Kadler K.E. (1996) Enzymic control of collagen
667 fibril shape. Journal of Molecular Biology 261, 93-97.

668 Holmes D.F., Watson R.B., Steinmann B. & Kadler K.E. (1993) Ehlers-Danlos syndrome
669 type VIIB. Morphology of type I collagen fibrils formed in vivo and in vitro is determined by
670 the conformation of the retained N-propeptide. Journal of Biological Chemistry 268,
671 15758-15765.

672 Huang G., Ge G., Wang D., Gopalakrishnan B., Butz D.H., Colman R.J., Nagy A. &
673 Greenspan D.S. (2011) alpha3(V) collagen is critical for glucose homeostasis in mice due
674 to effects in pancreatic islets and peripheral tissues. J Clin Invest 121, 769-783.

675 Hudson B.G., Tryggvason K., Sundaramoorthy M. & Neilson E.G. (2003) Alport's
676 syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 348, 2543-2556.

677 Hulmes D.J., Bruns R.R. & Gross J. (1983) On the state of aggregation of newly secreted
678 procollagen. Proceedings of the National Academy of Sciences of the United States of
679 America 80, 388-392.

680 Hulmes D.J., Miller A., Parry D.A., Piez K.A. & Woodhead-Galloway J. (1973) Analysis of
681 the primary structure of collagen for the origins of molecular packing. Journal of molecular
682 biology 79, 137-148.

683 Humphries S.M., Lu Y., Canty E.G. & Kadler K.E. (2008) Active negative control of
684 collagen fibrillogenesis in vivo. Intracellular cleavage of the type I procollagen propeptides
685 in tendon fibroblasts without intracellular fibrils. J Biol Chem 283, 12129-12135.

686 Huxley-Jones J., Robertson D.L. & Boot-Handford R.P. (2007) On the origins of the
687 extracellular matrix in vertebrates. Matrix biology : journal of the International Society for
688 Matrix Biology 26, 2-11.

689 Johnson C.D., Smith S.P. & Russell R.L. (1977) Electrophorus electricus
690 acetylcholinesterases; separation and selective modification by collagenase. J Neurochem
691 28, 617-624.

692 Johnstone I.L. (2000) Cuticle collagen genes. Expression in Caenorhabditis elegans. Trends
693 Genet 16, 21-27.

694 Jokinen J., Dadu E., Nykvist P., Kapyla J., White D.J., Ivaska J., Vehvilainen P., Reunanen
695 H., Larjava H., Hakkinen L. & Heino J. (2004) Integrin-mediated cell adhesion to type I
696 collagen fibrils. J Biol Chem 279, 31956-31963.

697 Kadler K.E., Baldock C., Bella J. & Boot-Handford R.P. (2007) Collagens at a glance.
698 Journal of Cell Science 120, 1955-1958.

699 Kadler K.E., Hojima Y. & Prockop D.J. (1987) Assembly of collagen fibrils de novo by
700 cleavage of the type I pC-collagen with procollagen C-proteinase. Assay of critical
701 concentration demonstrates that collagen self-assembly is a classical example of an
702 entropy-driven process. The Journal of biological chemistry 262, 15696-15701.

703 Kadler K.E., Hojima Y. & Prockop D.J. (1988) Assembly of type I collagen fibrils de novo.
704 Between 37 and 41 degrees C the process is limited by micro-unfolding of monomers. The
705 Journal of biological chemistry 263, 10517-10523.

706 Kadler K.E., Hojima Y. & Prockop D.J. (1990) Collagen fibrils in vitro grow from pointed
707 tips in the C- to N-terminal direction. The Biochemical journal 268, 339-343.

708 Kadler K.E., Holmes D.F., Graham H. & Starborg T. (2000) Tip-mediated fusion involving
709 unipolar collagen fibrils accounts for rapid fibril elongation, the occurrence of fibrillar
710 branched networks in skin and the paucity of collagen fibril ends in vertebrates. Matrix
711 biology : journal of the International Society for Matrix Biology 19, 359-365.

712 Kadler K.E., Torre-Blanco A., Adachi E., Vogel B.E., Hojima Y. & Prockop D.J. (1991) A
713 type I collagen with substitution of a cysteine for glycine-748 in the alpha 1(I) chain
714 copolymerizes with normal type I collagen and can generate fractallike structures.
715 Biochemistry 30, 5081-5088.

716 Kalamajski S. & Oldberg A. (2010) The role of small leucine-rich proteoglycans in collagen
717 fibrillogenesis. Matrix Biol 29, 248-253.

718 Kalson N.S., Holmes D.F., Herchenhan A., Lu Y., Starborg T. & Kadler K.E. (2011) Slow
719 stretching that mimics embryonic growth rate stimulates structural and mechanical
720 development of tendon-like tissue in vitro. Developmental dynamics : an official
721 publication of the American Association of Anatomists 240, 2520-2528.

722 Kalson N.S., Holmes D.F., Kapacee Z., Otermin I., Lu Y., Ennos R.A., Canty-Laird E.G. &
723 Kadler K.E. (2010) An experimental model for studying the biomechanics of embryonic
724 tendon: Evidence that the development of mechanical properties depends on the
725 actinomyosin machinery. Matrix Biology 29, 678-689.

726 Kalson N.S., Lu Y., Taylor S.H., Starborg T., Holmes D.F. & Kadler K.E. (2015) A
727 structure-based extracellular matrix expansion mechanism of fibrous tissue growth. Elife 4,
728 e05958.

729 Kalson N.S., Starborg T., Lu Y., Mironov A., Humphries S.M., Holmes D.F. & Kadler K.E.
730 (2013) Nonmuscle myosin II powered transport of newly formed collagen fibrils at the
731 plasma membrane. Proc Natl Acad Sci U S A 110, E4743-4752.

732 Kapacee Z., Richardson S.H., Lu Y., Starborg T., Holmes D.F., Baar K. & Kadler K.E.
733 (2008) Tension is required for fibripositor formation. Matrix Biology 27, 371-375.

734 Kapacee Z., Yeung C.Y., Lu Y., Crabtree D., Holmes D.F. & Kadler K.E. (2010) Synthesis
735 of embryonic tendon-like tissue by human marrow stromal/mesenchymal stem cells
736 requires a three-dimensional environment and transforming growth factor beta3. Matrix
737 Biology 29, 668-677.

738 Koch M., Laub F., Zhou P., Hahn R.A., Tanaka S., Burgeson R.E., Gerecke D.R., Ramirez F.
739 & Gordon M.K. (2003) Collagen XXIV, a vertebrate fibrillar collagen with structural
740 features of invertebrate collagens: selective expression in developing cornea and bone. J
741 Biol Chem 278, 43236-43244.

742 Kodama T., Freeman M., Rohrer L., Zabrecky J., Matsudaira P. & Krieger M. (1990) Type I
743 macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils.
744 Nature 343, 531-535.

745 Lees J.F., Tasab M. & Bulleid N.J. (1997) Identification of the molecular recognition
746 sequence which determines the type-specific assembly of procollagen. The EMBO journal
747 16, 908-916.

748 Legendre M., Santini S., Rico A., Abergel C. & Claverie J.M. (2011) Breaking the 1000-
749 gene barrier for Mimivirus using ultra-deep genome and transcriptome sequencing. Virol J
750 8, 99.

751 Li S.W., Prockop D.J., Helminen H., Fassler R., Lapvetelainen T., Kiraly K., Peltarri A.,
752 Arokoski J., Lui H., Arita M. & et al. (1995) Transgenic mice with targeted inactivation of
753 the Col2 alpha 1 gene for collagen II develop a skeleton with membranous and periosteal
754 bone but no endochondral bone. Genes Dev 9, 2821-2830.

755 Li S.W., Takanosu M., Arita M., Bao Y., Ren Z.X., Maier A., Prockop D.J. & Mayne R.
756 (2001) Targeted disruption of Col11a2 produces a mild cartilage phenotype in transgenic
757 mice: comparison with the human disorder otospondylomegaepiphyseal dysplasia
758 (OSMED). Dev Dyn 222, 141-152.

759 Li Y., Lacerda D.A., Warman M.L., Beier D.R., Yoshioka H., Ninomiya Y., Oxford J.T., Morris
760 N.P., Andrikopoulos K., Ramirez F. & et al. (1995) A fibrillar collagen gene, Col11a1, is
761 essential for skeletal morphogenesis. Cell 80, 423-430.

762 Lightfoot S.J., Holmes D.F., Brass A., Grant M.E., Byers P.H. & Kadler K.E. (1992) Type I
763 procollagens containing substitutions of aspartate, arginine, and cysteine for glycine in the

764 pro alpha 1 (I) chain are cleaved slowly by N-proteinase, but only the cysteine substitution
765 introduces a kink in the molecule. The Journal of biological chemistry 267, 25521-25528.

766 Liu C.Y., Birk D.E., Hassell J.R., Kane B. & Kao W.W. (2003) Keratocan-deficient mice
767 display alterations in corneal structure. J Biol Chem 278, 21672-21677.

768 Liu X., Wu H., Byrne M., Krane S. & Jaenisch R. (1997) Type III collagen is crucial for
769 collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci U
770 S A 94, 1852-1856.

771 Long K.B., Li Z., Burgwin C.M., Choe S.G., Martyanov V., Sassi-Gaha S., Earl J.P., Eutsey
772 R.A., Ahmed A., Ehrlich G.D., Artlett C.M., Whitfield M.L. & Blankenhorn E.P. (2015) The
773 Tsk2/+ mouse fibrotic phenotype is due to a gain-of-function mutation in the PIIINP
774 segment of the Col3a1 gene. J Invest Dermatol 135, 718-727.

775 Maass T., Bayley C.P., Morgelin M., Lettmann S., Bonaldo P., Paulsson M., Baldock C. &
776 Wagener R. (2016) Heterogeneity of Collagen VI Microfibrils: STRUCTURAL ANALYSIS OF
777 NON-COLLAGENOUS REGIONS. J Biol Chem 291, 5247-5258.

778 Maki J.M., Rasanen J., Tikkanen H., Sormunen R., Makikallio K., Kivirikko K.I. & Soininen
779 R. (2002) Inactivation of the lysyl oxidase gene Lox leads to aortic aneurysms,
780 cardiovascular dysfunction, and perinatal death in mice. Circulation 106, 2503-2509.

781 Mallory F.B. (1903) A Hitherto undescribed Fibrillar Substance produced by Connective-
782 Tissue Cells. J Med Res 10, 334-341.

783 Martin R., Farjanel J., Eichenberger D., Colige A., Kessler E., Hulmes D.J. & Giraud-Guille
784 M.M. (2000) Liquid crystalline ordering of procollagen as a determinant of three-
785 dimensional extracellular matrix architecture. Journal of molecular biology 301, 11-17.

786 McGuirt W.T., Prasad S.D., Griffith A.J., Kunst H.P., Green G.E., Shpargel K.B., Runge C.,
787 Huybrechts C., Mueller R.F., Lynch E., King M.C., Brunner H.G., Cremers C.W., Takanosu
788 M., Li S.W., Arita M., Mayne R., Prockop D.J., Van Camp G. & Smith R.J. (1999) Mutations
789 in COL11A2 cause non-syndromic hearing loss (DFNA13). Nat Genet 23, 413-419.

790 Mienaltowski M.J. & Birk D.E. (2014) Structure, physiology, and biochemistry of collagens.
791 Adv Exp Med Biol 802, 5-29.

792 Mussini E., Hutton J.J., Jr. & Udenfriend S. (1967) Collagen proline hydroxylase in wound
793 healing, granuloma formation, scurvy, and growth. Science 157, 927-929.

794 Myllyharju J. & Kivirikko K.I. (2004) Collagens, modifying enzymes and their mutations in
795 humans, flies and worms. Trends Genet 20, 33-43.

796 Njieha F.K., Morikawa T., Tuderman L. & Prockop D.J. (1982) Partial purification of a
797 procollagen C-proteinase. Inhibition by synthetic peptides and sequential cleavage of type
798 I procollagen. Biochemistry 21, 757-764.

799 Orgel J.P., San Antonio J.D. & Antipova O. (2011) Molecular and structural mapping of
800 collagen fibril interactions. Connective tissue research 52, 2-17.

801 Parry D.A., Barnes G.R. & Craig A.S. (1978) A comparison of the size distribution of
802 collagen fibrils in connective tissues as a function of age and a possible relation between
803 fibril size distribution and mechanical properties. Proc R Soc Lond B Biol Sci 203, 305-321.

804 Petropoulou V., Garrigue-Antar L. & Kadler K.E. (2005) Identification of the minimal
805 domain structure of bone morphogenetic protein-1 (BMP-1) for chordinase activity:
806 chordinase activity is not enhanced by procollagen C-proteinase enhancer-1 (PCPE-1). The
807 Journal of biological chemistry 280, 22616-22623.

808 Pingel J., Lu Y., Starborg T., Fredberg U., Langberg H., Nedergaard A., Weis M., Eyre D.,
809 Kjaer M. & Kadler K.E. (2014) 3-D ultrastructure and collagen composition of healthy and
810 overloaded human tendon: evidence of tenocyte and matrix buckling. J Anat 224, 548-
811 555.

812 Plumb D.A., Dhir V., Mironov A., Ferrara L., Poulsom R., Kadler K.E., Thornton D.J., Briggs
813 M.D. & Boot-Handford R.P. (2007) Collagen XXVII is developmentally regulated and forms
814 thin fibrillar structures distinct from those of classical vertebrate fibrillar collagens. J Biol
815 Chem 282, 12791-12795.

816 Plumb D.A., Ferrara L., Torbica T., Knowles L., Mironov A., Jr., Kadler K.E., Briggs M.D. &
817 Boot-Handford R.P. (2011) Collagen XXVII organises the pericellular matrix in the growth
818 plate. PloS one 6, e29422.

819 Rasmussen M., Jacobsson M. & Bjorck L. (2003) Genome-based identification and analysis
820 of collagen-related structural motifs in bacterial and viral proteins. J Biol Chem 278,
821 32313-32316.

822 Reid K.B. & Day A.J. (1990) Ig-binding domains of C1q. Immunol Today 11, 387-388.

823 Richardson S.H., Starborg T., Lu Y., Humphries S.M., Meadows R.S. & Kadler K.E. (2007)
824 Tendon development requires regulation of cell condensation and cell shape via cadherin-
825 11-mediated cell-cell junctions. Molecular and Cellular biology 27, 6218-6228.

826 Roberts A.B., Sporn M.B., Assoian R.K., Smith J.M., Roche N.S., Wakefield L.M., Heine
827 U.I., Liotta L.A., Falanga V., Kehrl J.H. & et al. (1986) Transforming growth factor type
828 beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen
829 formation in vitro. Proc Natl Acad Sci U S A 83, 4167-4171.

Saito K., Chen M., Bard F., Chen S., Zhou H., Woodley D., Polischuk R., Schekman R. & Malhotra V. (2009) TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. Cell 136, 891-902.

Satoh M., Hirayoshi K., Yokota S., Hosokawa N. & Nagata K. (1996) Intracellular interaction of collagen-specific stress protein HSP47 with newly synthesized procollagen. J Cell Biol 133, 469-483.

Silver F.H. & Trelstad R.L. (1980) Type I collagen in solution. Structure and properties of fibril fragments. J Biol Chem 255, 9427-9433.

Starborg T., Kalson N.S., Lu Y., Mironov A., Cootes T.F., Holmes D.F. & Kadler K.E. (2013) Using transmission electron microscopy and 3View to determine collagen fibril size and three-dimensional organization. Nature protocols 8, 1433-1448.

Stearns M.L. (1940) Studies on the development of connective tissue in transparent chambers in the rabbit's ear. II - Stearns - 2005 - American Journal of Anatomy - Wiley Online Library. American Journal of Anatomy.

Svensson L., Aszodi A., Reinholt F.P., Fassler R., Heinegard D. & Oldberg A. (1999) Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. The Journal of biological chemistry 274, 9636-9647.

Sweeney S.M., Orgel J.P., Fertala A., McAuliffe J.D., Turner K.R., Di Lullo G.A., Chen S., Antipova O., Perumal S., Ala-Kokko L., Forlino A., Cabral W.A., Barnes A.M., Marini J.C. & San Antonio J.D. (2008) Candidate cell and matrix interaction domains on the collagen fibril, the predominant protein of vertebrates. The Journal of biological chemistry 283, 21187-21197.

Takaluoma K., Hyry M., Lantto J., Sormunen R., Bank R.A., Kivirikko K.I., Myllyharju J. & Soininen R. (2007) Tissue-specific changes in the hydroxylysine content and cross-links of collagens and alterations in fibril morphology in lysyl hydroxylase 1 knock-out mice. J Biol Chem 282, 6588-6596.

Tasheva E.S., Koester A., Paulsen A.Q., Garrett A.S., Boyle D.L., Davidson H.J., Song M., Fox N. & Conrad G.W. (2002) Mimecan/osteoglycin-deficient mice have collagen fibril abnormalities. Mol Vis 8, 407-415.

Taylor S.H., Yeung C.Y., Kalson N.S., Lu Y., Zigrino P., Starborg T., Warwood S., Holmes D.F., Canty-Laird E.G., Mauch C. & Kadler K.E. (2015) Matrix metalloproteinase 14 is required for fibrous tissue expansion. Elife 4, e09345.

Thorpe C.T., Streeter I., Pinchbeck G.L., Goodship A.E., Clegg P.D. & Birch H.L. (2010) Aspartic acid racemization and collagen degradation markers reveal an accumulation of damage in tendon collagen that is enhanced with aging. J Biol Chem 285, 15674-15681.

865 Thurmond F.A. & Trotter J.A. (1994) Native collagen fibrils from echinoderms are
866 molecularly bipolar. J Mol Biol 235, 73-79.

867 Trelstad R.L. & Birk D.E. (1985) The fibroblast in morphogenesis and fibrosis: cell
868 topography and surface-related functions. Ciba Found Symp 114, 4-19.

869 Trelstad R.L. & Hayashi K. (1979) Tendon collagen fibrillogenesis: intracellular
870 subassemblies and cell surface changes associated with fibril growth. Developmental
871 biology 71, 228-242.

872 Trotter J.A., Chapman J.A., Kadler K.E. & Holmes D.F. (1998) Growth of sea cucumber
873 collagen fibrils occurs at the tips and centers in a coordinated manner. J Mol Biol 284,
874 1417-1424.

875 Trotter J.A., Kadler K.E. & Holmes D.F. (2000) Echinoderm collagen fibrils grow by
876 surface-nucleation-and-propagation from both centers and ends. J Mol Biol 300, 531-540.

877 Trotter J.A. & Koob T.J. (1989) Collagen and proteoglycan in a sea urchin ligament with
878 mutable mechanical properties. Cell Tissue Res 258, 527-539.

879 Tuderman L. & Prockop D.J. (1982) Procollagen N-proteinase. Properties of the enzyme
880 purified from chick embryo tendons. European journal of biochemistry / FEBS 125, 545-
881 549.

882 Vandenberg P., Khillan J.S., Prockop D.J., Helminen H., Kontusaari S. & Ala-Kokko L.
883 (1991) Expression of a partially deleted gene of human type II procollagen (COL2A1) in
884 transgenic mice produces a chondrodysplasia. Proc Natl Acad Sci U S A 88, 7640-7644.

885 Venditti R., Scanu T., Santoro M., Di Tullio G., Spaar A., Gaibisso R., Beznoussenko G.V.,
886 Mironov A.A., Mironov A., Jr., Zelante L., Piemontese M.R., Notarangelo A., Malhotra V.,
887 Vertel B.M., Wilson C. & De Matteis M.A. (2012) Sedlin controls the ER export of
888 procollagen by regulating the Sar1 cycle. Science 337, 1668-1672.

889 Verzijl N., DeGroot J., Thorpe S.R., Bank R.A., Shaw J.N., Lyons T.J., Bijlsma J.W., Lafeber
890 F.P., Baynes J.W. & TeKoppele J.M. (2000) Effect of collagen turnover on the
891 accumulation of advanced glycation end products. J Biol Chem 275, 39027-39031.

892 Vogel B.E., Doelz R., Kadler K.E., Hojima Y., Engel J. & Prockop D.J. (1988) A substitution
893 of cysteine for glycine 748 of the alpha 1 chain produces a kink at this site in the
894 procollagen I molecule and an altered N-proteinase cleavage site over 225 nm away. The
895 Journal of biological chemistry 263, 19249-19255.

896 Wallis G.A., Kadler K.E., Starman B.J. & Byers P.H. (1992) A tripeptide deletion in the
897 triple-helical domain of the pro alpha 1(I) chain of type I procollagen in a patient with

lethal osteogenesis imperfecta does not alter cleavage of the molecule by N-proteinase. The Journal of biological chemistry 267, 25529-25534.

Watson R.B., Holmes D.F., Graham H.K., Nusgens B.V. & Kadler K.E. (1998) Surface located procollagen N-propeptides on dermatosparactic collagen fibrils are not cleaved by procollagen N-proteinase and do not inhibit binding of decorin to the fibril surface. Journal of Molecular Biology 278, 195-204.

Watson R.B., Wallis G.A., Holmes D.F., Viljoen D., Byers P.H. & Kadler K.E. (1992) Ehlers Danlos syndrome type VIIB. Incomplete cleavage of abnormal type I procollagen by N-proteinase in vitro results in the formation of copolymers of collagen and partially cleaved pNcollagen that are near circular in cross-section. Journal of Biological Chemistry 267, 9093-9100.

Wenstrup R.J., Florer J.B., Brunskill E.W., Bell S.M., Chervoneva I. & Birk D.E. (2004) Type V collagen controls the initiation of collagen fibril assembly. J Biol Chem 279, 53331-53337.

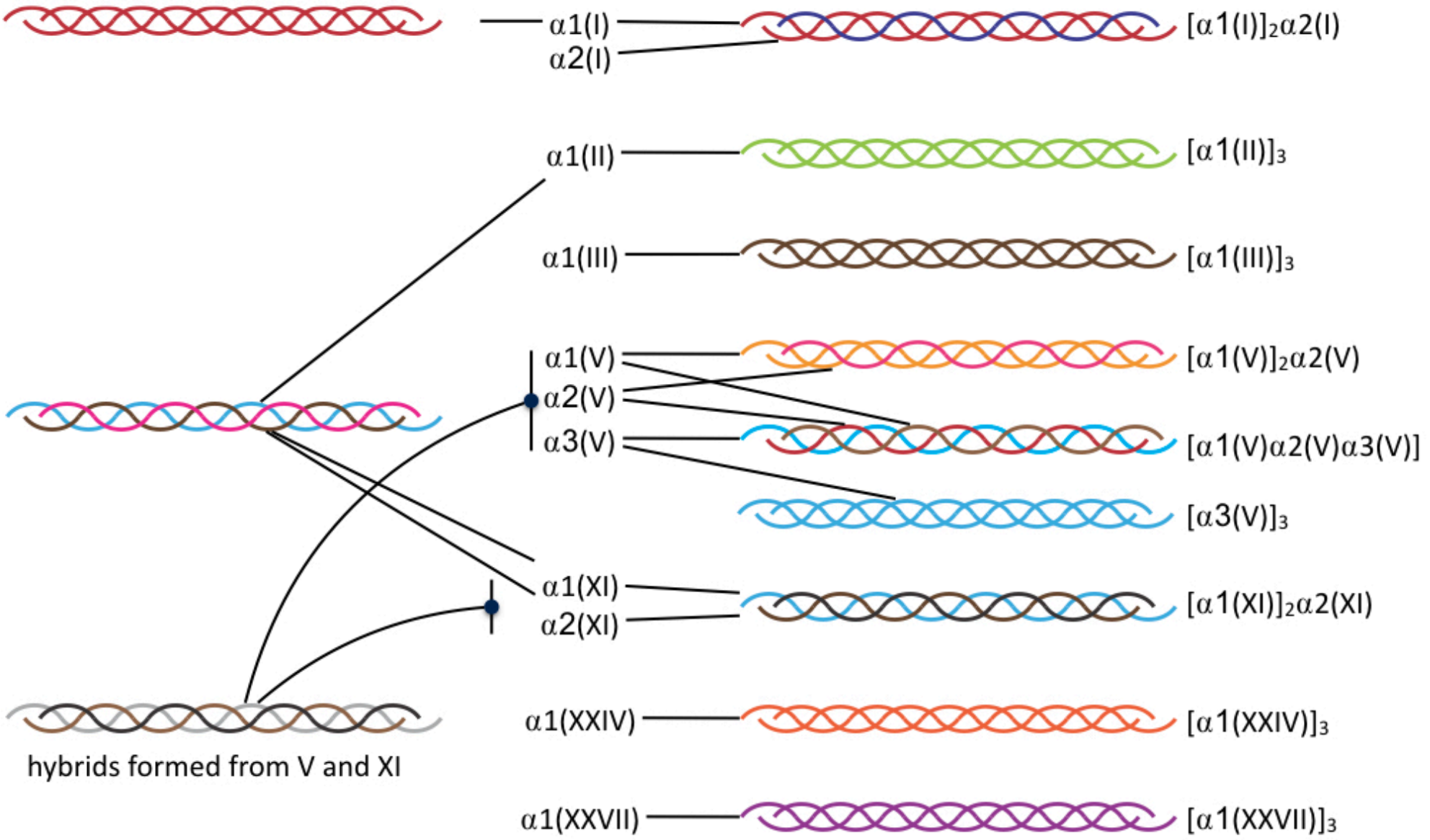
Wilson D.G., Phamluong K., Li L., Sun M., Cao T.C., Liu P.S., Modrusan Z., Sandoval W.N., Rangell L., Carano R.A., Peterson A.S. & Solloway M.J. (2011) Global defects in collagen secretion in a Mia3/TANGO1 knockout mouse. J Cell Biol 193, 935-951.

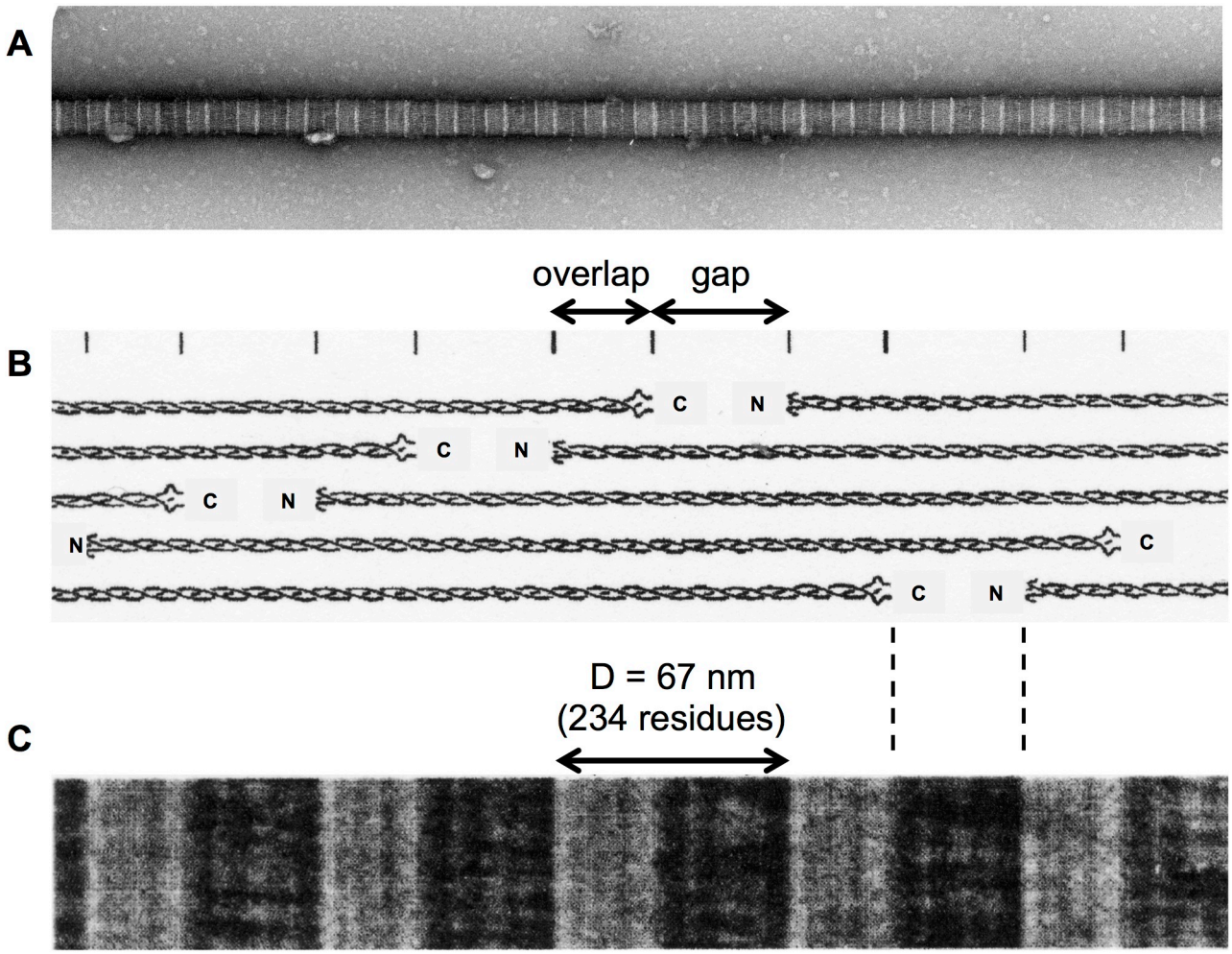
Wood G.C. & Keech M.K. (1960) The formation of fibrils from collagen solutions. 1. The effect of experimental conditions: kinetic and electron-microscope studies. Biochem J 75, 588-598.

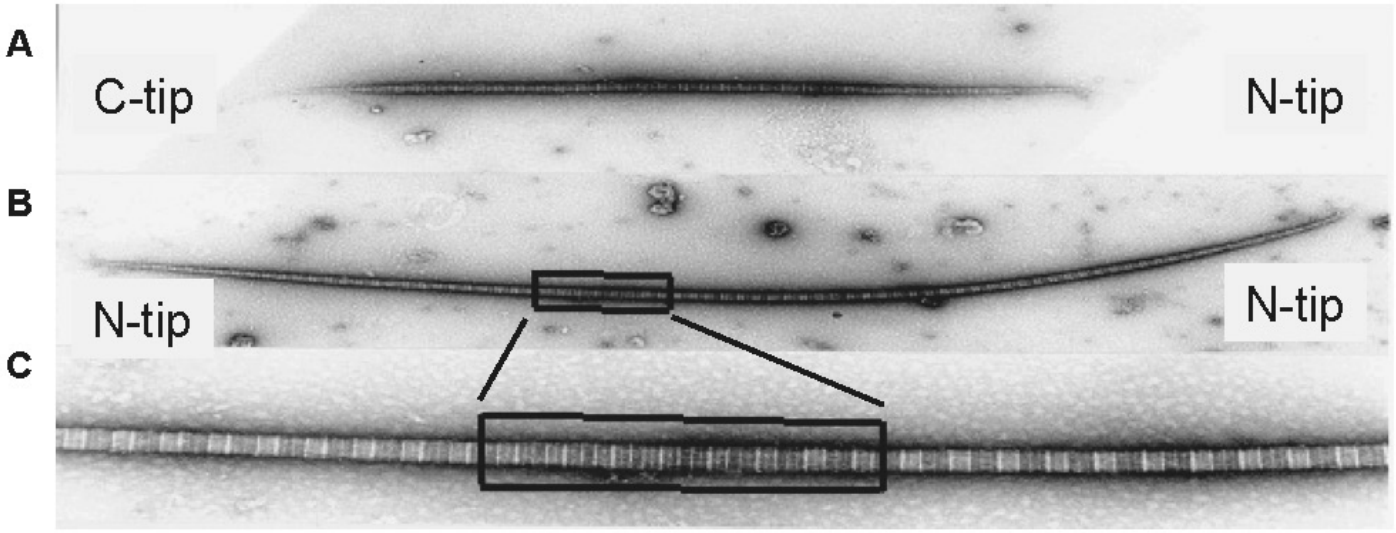
Yeung C.Y., Gossan N., Lu Y., Hughes A., Hensman J.J., Bayer M.L., Kjaer M., Kadler K.E. & Meng Q.J. (2014) Gremlin-2 is a BMP antagonist that is regulated by the circadian clock. Scientific reports 4, 5183.

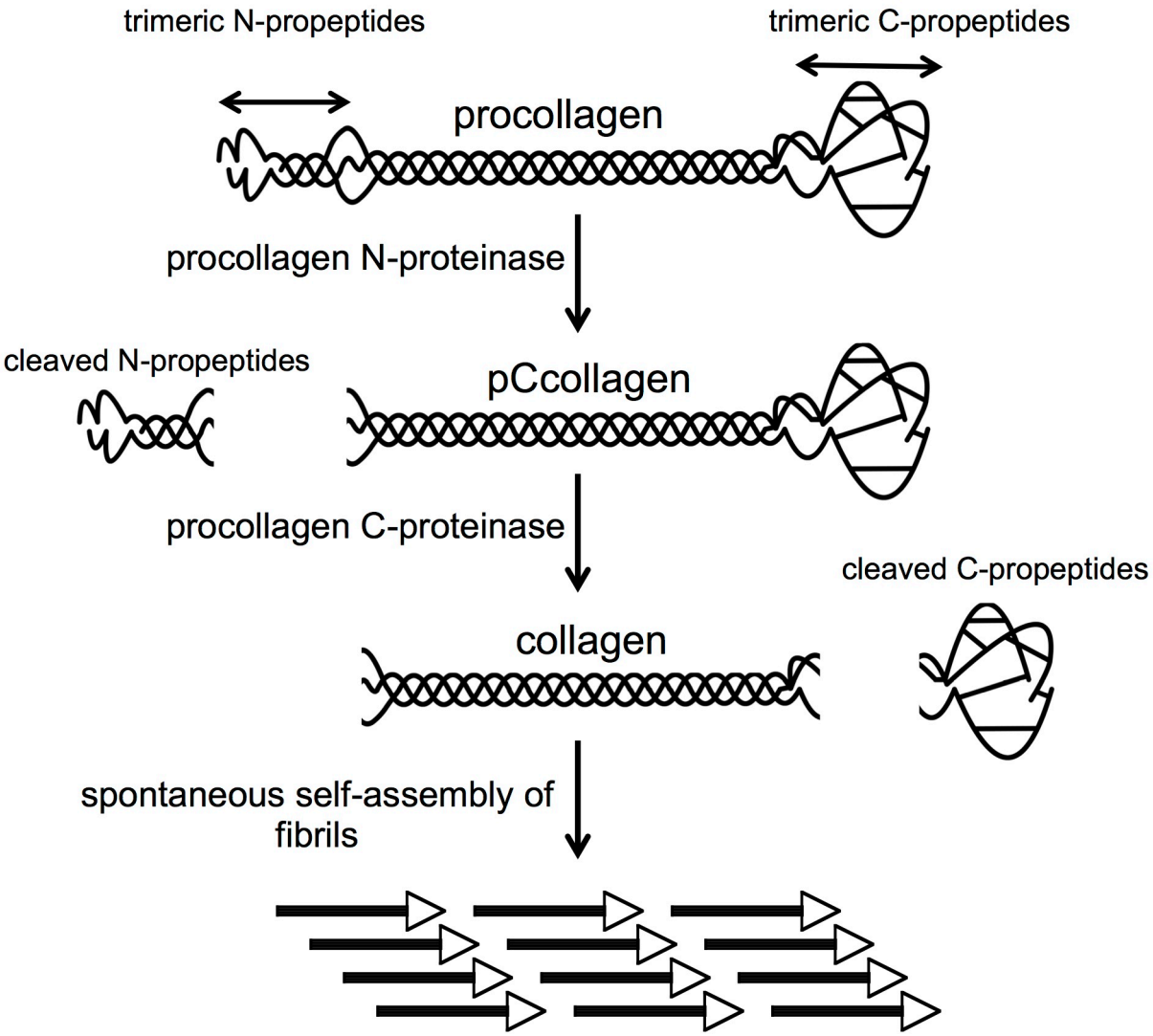
Young B.B., Zhang G., Koch M. & Birk D.E. (2002) The roles of types XII and XIV collagen in fibrillogenesis and matrix assembly in the developing cornea. J Cell Biochem 87, 208-220.

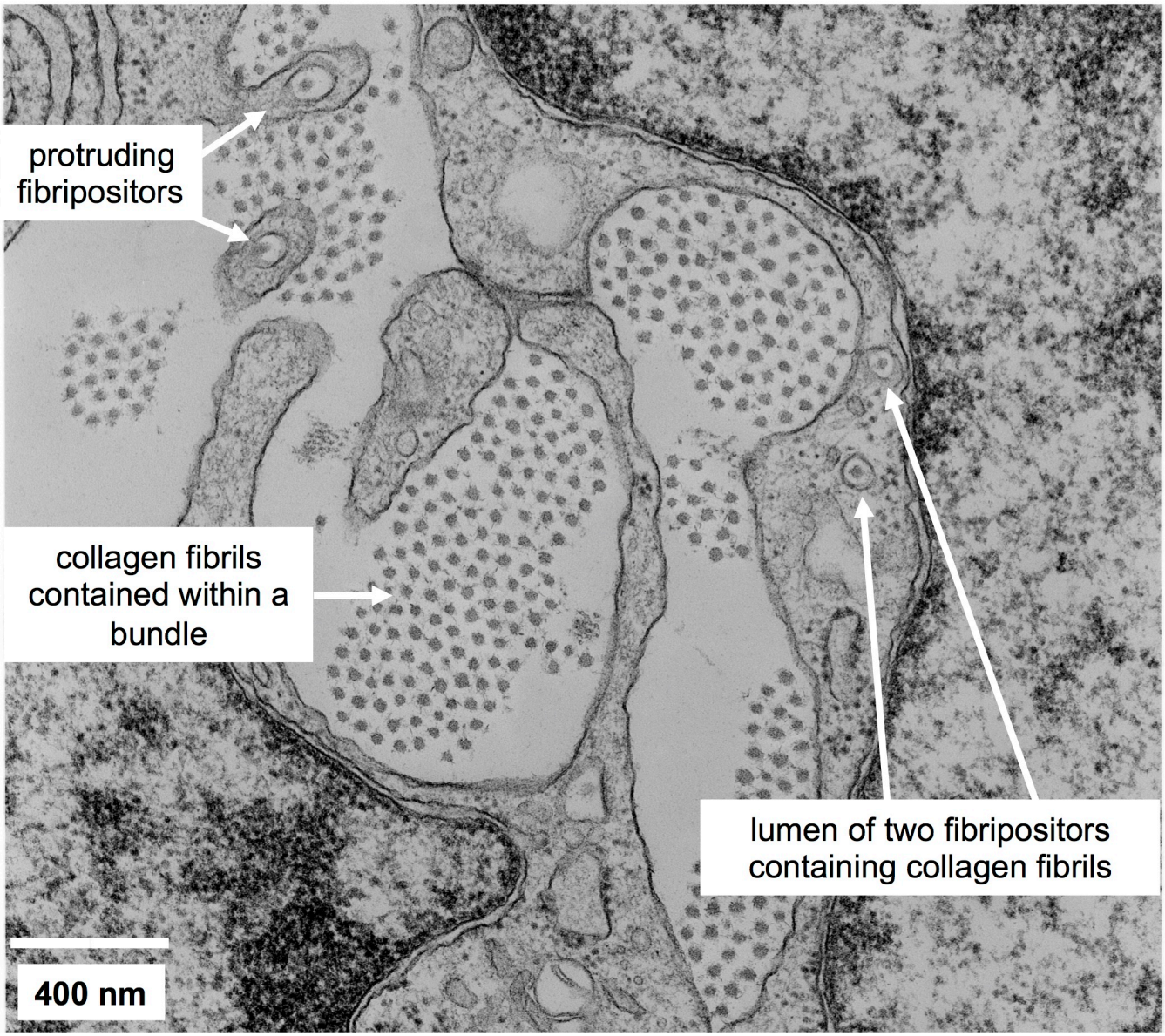
Young R.D., Knupp C., Pinali C., Png K.M., Ralphs J.R., Bushby A.J., Starborg T., Kadler K.E. & Quantock A.J. (2014) Three-dimensional aspects of matrix assembly by cells in the developing cornea. Proc Natl Acad Sci U S A 111, 687-692.

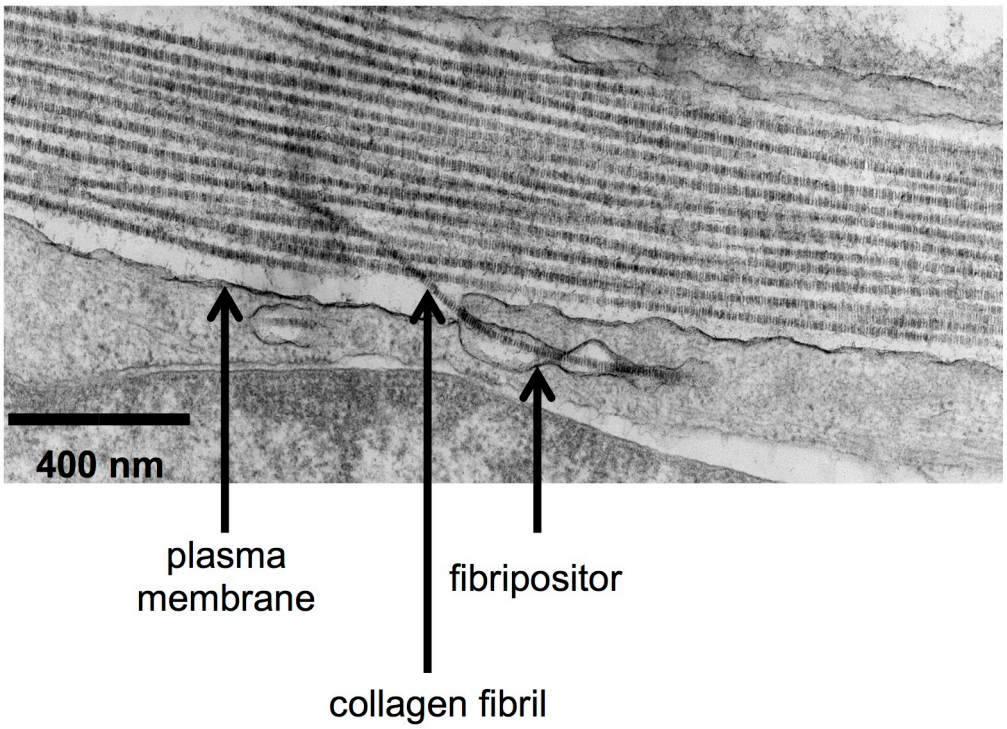

α1(I)
α2(I)
[α1(I)]2α2(I)
α1(II)
[α1(II)]3
α1(III)
[α1(III)]3
α1(V)
α2(V)
α3(V)
[α1(V)]2α2(V)
[α1(V)α2(V)α3(V)]
[α3(V)]3
α1(XI)
α2(XI)
[α1(XI)]2α2(XI)
α1(XXIV)
[α1(XXIV)]3
α1(XXVII)
[α1(XXVII)]3
hybrids formed from V and XI

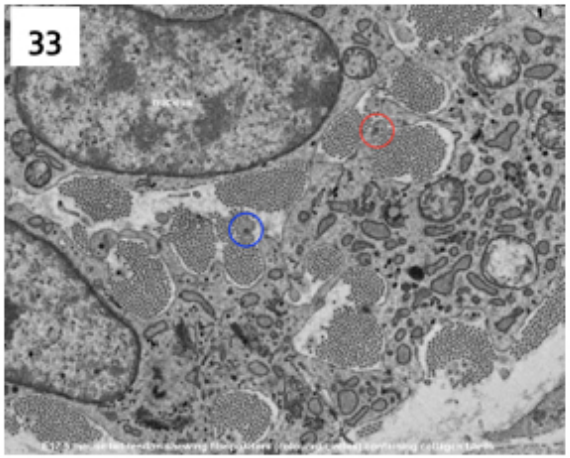

A
overlap
gap
B
C
N
D = 67 nm
(234 residues)
C

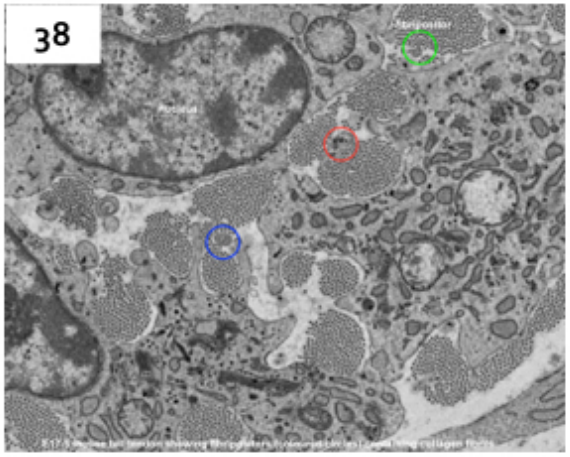

A
C-tip
N-tip
B
N-tip
N-tip
C

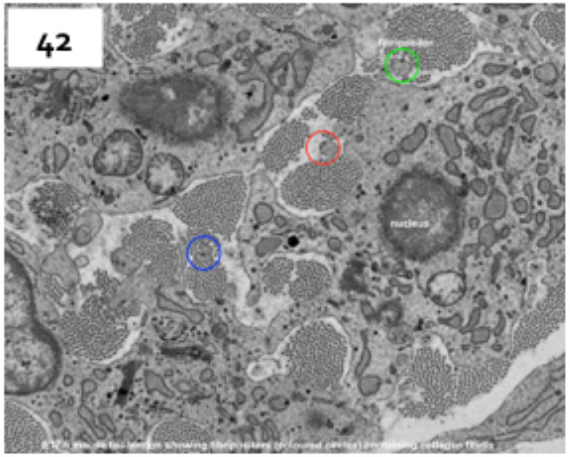

trimeric N-propeptides
trimeric C-propeptides
procollagen
procollagen N-proteinase
cleaved N-propeptides
pCcollagen
procollagen C-proteinase
cleaved C-propeptides
collagen
spontaneous self-assembly of fibrils


protruding fibripositors
collagen fibrils contained within a bundle
lumen of two fibripositors containing collagen fibrils
400 nm


400 nm
plasma
membrane
fibripositor
collagen fibril


33


38


42