ANNUAL REVIEWS

Annual Review of Biochemistry

Quality Control of Procollagen in Cells

Shinya Ito[1] and Kazuhiro Nagata[1,2,3]

[1]Faculty of Life Sciences, Kyoto Sangyo University, Kyoto 603-8555, Japan; email: s.ito@cc.kyoto-su.ac.jp

[2]Institute for Protein Dynamics, Kyoto Sangyo University, Kyoto 603-8555, Japan; email: nagata@cc.kyoto-su.ac.jp

[3]JT Biohistory Research Hall, Osaka, 569-1125, Japan

Annu. Rev. Biochem. 2021. 90:631–58

First published as a Review in Advance on April 6, 2021

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-013118-111603

Keywords

collagen, triple-helix structure, molecular chaperone Hsp47, quality control, ER-phagy

Abstract

Collagen is the most abundant protein in mammals. A unique feature of collagen is its triple-helical structure formed by the Gly-Xaa-Yaa repeats. Three single chains of procollagen make a trimer, and the triple-helical structure is then folded in the endoplasmic reticulum (ER). This unique structure is essential for collagen's functions in vivo, including imparting bone strength, allowing signal transduction, and forming basement membranes. The triple-helical structure of procollagen is stabilized by post-translational modifications and intermolecular interactions, but collagen is labile even at normal body temperature. Heat shock protein 47 (Hsp47) is a collagen-specific molecular chaperone residing in the ER that plays a pivotal role in collagen biosynthesis and quality control of procollagen in the ER. Mutations that affect the triple-helical structure or result in loss of Hsp47 activity cause the destabilization of procollagen, which is then degraded by autophagy. In this review, we present the current state of the field regarding quality control of procollagen.

Contents

1. INTRODUCTION

Collagen is the most abundant protein in mammals, comprising approximately one-third of total protein (1). The tissues with the highest collagen contents in the mammalian body are tendons (80% collagen content based on dry, fat-free weight), skin (70%), bones (25%), and the aorta (20%) (1). The timing of expression of the different types of collagens varies (2); type IV collagen, a component of the basement membrane, is required at 10 to 11 days post coitum (dpc) in mice (3), but the expression of type I collagen is observed much earlier (4). In general, collagen is the major component of the extracellular matrix (ECM), where it forms a specialized network around cells and is essential for cell–cell interactions and cell attachment to the basement membrane. To date, 28 different types of collagen have been identified in mammalian cells, all sharing a common structural feature (5), namely, a triple-helical domain composed of the three–amino acid repeat Gly-Xaa-Yaa. Collagens can be categorized based on which of the numerous distinct collagen superstructures with diverse biological activities they form, including fibril-forming collagens (I, II, III, V, XI, XXIV, and XXVII), fibril-associated collagens with interrupted triple helices (IX, XII, XIV, XVI, XIX, XX, XXI, and XXII), network-forming collagens (IV, VIII, and X), beaded filament–forming collagens (VI), anchoring fibril–forming collagens (VII), membrane collagens (XIII, XVII, XXIII, and XXV), and multiplexins (XV and XVIII).

Type I collagen is a typical fibril-forming collagen consisting of two α1 chains and one α2 chain, which are cotranslationally inserted into the endoplasmic reticulum (ER). Proline at the

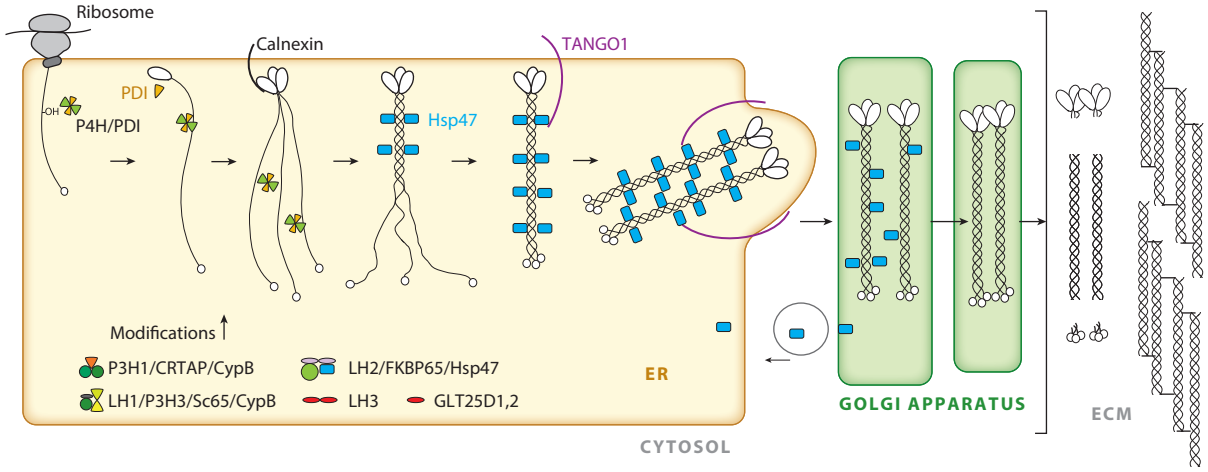


Figure 1

Procollagen folding in the cell. Nascent procollagen chains are co-translationally translocated into the ER. Proline residues at the Yaa position of the Gly-Xaa-Yaa repeat in procollagen are co-translationally and posttranslationally hydroxylated by the P4H–PDI complex. LH complexes hydroxylate some specific lysine residues. Some hydroxylated lysines are further glycosylated by LH3 and/or GLTs. Prolyl hydroxylation and lysyl hydroxylation are required for the stability of the triple-helix structure and the crosslinking of mature collagen outside of the cell, respectively. The C-propeptide domains associate and form a trimer via disulfide bonding. ER membrane lectin-like molecular chaperone, calnexin, and the ER oxidoreductase PDI are involved in this step. The triple helical conformation is formed from the C terminus to the N terminus. The collagen-specific molecular chaperone Hsp47 interacts with the triple-helical procollagen and prevents local procollagen unfolding and aggregate formation. Procollagen with bound Hsp47 is recognized by TANGO1, an ER exit site organizer for mega-carrier trafficking to the *cis*-Golgi apparatus, where Hsp47 dissociates and returns to the ER by binding with the KDEL(REDL) receptor. Procollagen is secreted out of the cell via the Golgi apparatus. N and C propeptidases cleave the N and C propeptides, respectively. Mature collagen then assembles into fibers via crosslinking. Abbreviations: CRTAP, cartilage-associated protein; CypB, cyclophilin B; ECM, extracellular matrix; ER, endoplasmic reticulum; GLT, glycosyltransferase; Hsp47, heat shock protein 47; LH, lysyl hydroxylase; P3H, prolyl 3-hydroxylase; P4H, prolyl 4-hydroxylase; PDI, protein disulfide isomerase; Sc65, synaptonemal complex 65; TANGO1, transport and Golgi organization 1. Figure adapted with permission from Reference 7.

Yaa position is hydroxylated by prolyl 4-hydroxylase (P4H). Once two α1 chains and one α2 chain assemble and form interchain disulfide bonds between their C propeptide regions, then triple-helix formation proceeds from the C terminus to the N terminus in a zipper-like fashion (6). A correctly folded procollagen is transported from the ER to the cell surface via the Golgi apparatus (**Figure 1**). When the procollagen molecule reaches the outer surface of the cell, its N and C propeptides are cleaved off by N and C propeptidases, respectively; this is followed by crosslinking and the formation of collagen bundles in a staggered manner in the ECM.

The defining feature of collagen is its molecular structure, i.e., the unique supercoiled triple helix in which three parallel polypeptide strands coil around each other in a left-handed, polyproline II–type helical conformation with a one-residue stagger, forming a right-handed triple helix (**Figure 2*a***). The correctly folded triple-helical structure is essential for the functions of collagen in the ECM, including cell–cell interactions and signaling from the external environment (8). Because the triple-helical structure is sensitive to heat, type I collagen is thermally unstable even at body temperature (9). Disruption of the triple-helical structure leads to collagen-related diseases, including osteogenesis imperfecta (OI) (10) and Ehlers–Danlos syndrome (11). In this review, we focus on procollagen folding in the cell and the maintenance of procollagen quality control. Once the quality-control mechanism is overwhelmed, various collagen-related diseases develop in various tissues, and these are also summarized in this review.

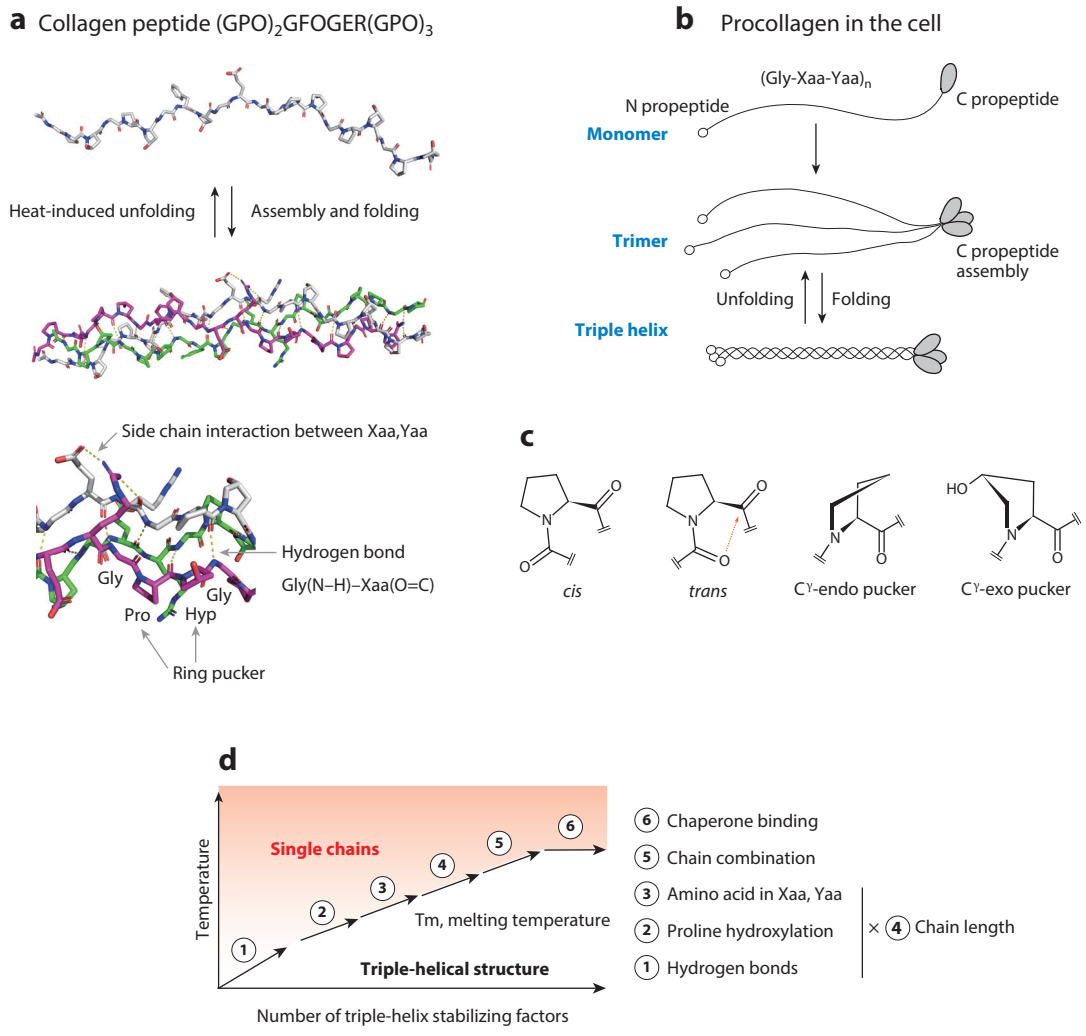


Figure 2

Formation of the triple-helical structure of collagen. (*a*) The collagen peptide consisting of multiple Gly-Xaa-Yaa repeats is folded into a triple-helical structure below its melting temperature and is unfolded at high temperatures (Protein Data Bank identifier: 1Q7D). The triple-helical structure is stabilized by hydrogen bonds between a glycine N–H and the backbone carbonyl (O=C) at the Xaa position in an adjacent chain. Proline at the Yaa position is hydroxylated, which changes the orientation of the proline ring. (*b*) In cells, monomers of procollagen form trimers via the C-propeptide region before triple-helix formation. The triple helix is formed from the C terminus to the N terminus in a zipper-like manner. (*c*) Proline *cis* and *trans* forms. The proline ring has both Cγ-endo and Cγ-exo pucker orientations. Proline and hydroxyproline favor the Cγ-endo and Cγ-exo pucker forms, respectively. (*d*) The collagen triple helix is thermally stabilized by hydrogen bonds, proline hydroxylation, the amino acids at the Xaa and Yaa positions, chain length, the combination of α-chains, and a combination of all of these factors. The above factors raise the melting temperature of the collagen peptide. In vivo, chaperone binding is also critical for collagen stability.

2. COLLAGEN AS A TRIPLE-HELICAL STRUCTURE

Numerous studies of the Gly-Xaa-Yaa–repeat collagen peptide have revealed stabilizing factors for the triple-helical structure. The triple helix has a characteristic circular dichroism spectrum, with a maximum near 225 nm and a minimum near 198 nm, which reflects the conformation of

polyproline II, three strands of which are supercoiled around a common axis (**Figure 2*a***) (12). Thermal stability can be measured by observing this typical circular dichroism spectrum and tracking the average residual ellipticity at 225 nm with increasing temperature. The melting temperature is the best indicator of the stability of the triple-helical structure composed of Gly-Xaa-Yaa–repeat peptides (13).

2.1. Interstrand Hydrogen Bonds with Glycine in Gly-Xaa-Yaa Repeats

An amide-amide hydrogen bond forms between a glycine N–H and the backbone carbonyl (O=C) of the amino acid in the Xaa position of an adjacent chain (**Figure 2*a***). The glycine residues are buried within the central core of the triple helix and are solvent inaccessible, while the residues in the Xaa or Yaa position are highly exposed to the solvent because of their proximity to the neighboring chain (14). The importance of glycine for triple-helix stability is supported by the observation that glycine substitution in type I collagen destabilizes the triple-helical structure (9), resulting in OI (15).

2.2. Stereoelectronic Effects of Proline Hydroxylation on Yaa

The amino acids in the Xaa and Yaa positions of collagen are often proline (28.1%) and hydroxyproline (Hyp; 38.1%), respectively (16). The most common triplet is Gly-Pro-Hyp (10.5%). Almost all proline residues in the Yaa position of a procollagen monomer are hydroxylated by P4H. Proline and hydroxyproline preorganize the individual strands into a polyproline II conformation, thereby decreasing the entropic cost of collagen folding. Two ring conformations of proline and its derivatives exist, C^{γ}-endo pucker and C^{γ}-exo pucker (**Figure 2*c***). The C^{γ}-exo conformation is strongly favored by stereoelectronic effects when proline is hydroxylated (17). The $n \rightarrow \pi^*$ interaction occurs when the oxygen of a peptide bond donates electron density from its lone pairs into the empty π^* orbital of a nearby carbonyl group in the neighboring peptide chain (18). Only when the proline residue is in the C^{γ}-exo ring pucker can an $n \rightarrow \pi^*$ interaction stabilize the *trans* isomer of the peptide bond. The C^{γ}-exo ring pucker preorganizes the main chain torsion angles and enhances triple-helix stability (19). Hence, prolyl 4-hydroxylation is essential for the stability of triple-helical collagen due to its a stereoelectronic effect.

Proline exists in *cis* and *trans* conformations. The nascent chain is translated with proline in the *cis* form and folded with proline in the *trans* form (**Figure 2*c***). Because collagen is proline-rich, proline *cis-trans* isomerization is important for the folding of the triple helix. However, the involvement of peptidyl-prolyl isomerases (PPIases), including FKBP22, FKBP65, and cyclophilin B (CypB), in proline *cis-trans* isomerization in the ER is not yet clear, because each PPIase forms a complex with modification enzymes for procollagens (see Sections 3.2–3.4), making it difficult to distinguish between its modification effect and its role as a PPIase.

Sequence-dependence of the triple-helix pitch is well established, with imino acid–rich sequences forming a helical repeat with seven residues per two turns (7/2) and imino acid–poor sequences forming a looser helical repeat with ten residues per three turns (10/3) (20, 21). The local domain of the Gly-Xaa-Yaa repeat and the native collagen type can affect the actual helical pitch of collagen. Furthermore, the variability in the triple-helical pitch of native collagen can play a role in interactions between collagenous domains and other biomolecules, including matrix metalloproteinase (MMP) 1 (22), integrin (23), and fibronectin (24).

2.3. Side-Chain Interactions of the Xaa and Yaa Residues

Although stereoelectronic effects and preorganization by proline hydroxylation play key roles in triple-helix stability, the amino acids in the Xaa and Yaa positions are not always proline. Based

on calculations from type I, II, III, V, and XI collagens, the preferences of different amino acids for the Xaa and Yaa positions were reported. Cysteine and tryptophan residues are not found at either the Xaa or Yaa position (16). Tyrosine residues are also not found at the Yaa position in these collagens. Residues in the Xaa position are somewhat more exposed than those in the Yaa position. Side chains of the Xaa and Yaa residues interact with the main chain or with the side chains, and these interactions contribute to triple-helix stability. Importantly, the presence of particular amino acids in positions Xaa and Yaa is tightly correlated with the stability of the local triple-helical conformation (16). Proline (32.9%), glutamine (13%), and alanine (11.1%) are often found in the Xaa position, and hydroxyproline (34%), arginine (11.4%), and alanine (10.6%) in the Yaa position. Salt bridges between arginine and lysine and between aspartate and glutamate are observed in the crystal structure of collagen peptide (25) and contribute to the stability of the triple-helix structure.

2.4. Other Factors Affecting the Stability of Collagen Peptides

The stability of the collagen Gly-Xaa-Yaa peptide depends on the chain length. The most stabilizing triplet is Pro-Hyp-Gly, and $(Pro\text{-}Hyp\text{-}Gly)_6$ is the minimum length required for stable triple-helix formation. For some collagen types, there are homotrimeric (types II, III, X, XIII, etc.) and heterotrimeric (types I, IV, V, VI, etc.) forms. Type I collagen is composed of two α1 chains and one α2 chain. Type V collagen is composed of α1, α2, and α3 chains. Of note, heterotrimers are more stable than homotrimers, because Coulomb interactions between a negatively charged and a positively charged chain stabilize the triple helix (26).

In addition to posttranslational modifications, the chain combination, the temperature, and the presence of collagen-specific molecular chaperones are critical factors for stabilizing the collagen triple helix in vivo; these factors are described in Sections 3–5 of this review. The triple-helical conformation of collagen can be evaluated by nuclear magnetic resonance and circular dichroism in vitro and by protease sensitivity and collagen-hybridizing peptide assays in vivo (27). Native triple-helical structures are resistant to digestion by trypsin, chymotrypsin, pepsin, and other common proteases, and these enzymes can be used as a convenient method for examining triple-helix formation in vivo.

3. POSTTRANSLATIONAL MODIFICATIONS

Procollagen is modified cotranslationally and posttranslationally by enzyme families including procollagen P4Hs, prolyl 3-hydroxylases (P3Hs), lysyl hydroxylases (LHs), *O*-glycosyltransferases, and *N*-glycosyltransferases. Generally, these modifications can be added only to prefolded single chains before triple-helix formation (**Figure 1**). However, when the temperature exceeds body temperature in vitro, the folded triple-helical procollagen becomes loose and is overmodified in the ER. Type I collagen has only one *N*-glycosylation site in the C propeptide region. Recently, calnexin, an ER-resident membrane lectin, was reported to recognize this *N*-glycan in C propeptide and be involved in the quality control of procollagen (28), as described in Section 7. Knockout (KO) or mutation of procollagen-modifying enzymes results in collagen-related phenotypes in vivo, as summarized in **Table 1**.

3.1. Prolyl 4-Hydroxylase–Protein Disulfide Isomerase

Procollagen P4Hs play a central role in the biosynthesis of collagen because 4-hydroxyproline residues are essential for stabilization of the collagen triple-helix structure. P4Hs catalyze the

Table 1 Collagen synthesis–related proteins in vertebrates

Role	Protein	Gene	Typical substrate	Phenotypes of KO mice and cells	Human disease caused by mutation[a]	Collagen-related disease conditions associated with upregulation
Prolyl 4-hydroxylase	P4Ha1	*P4ha1*	$(GPP)_n$ single chain	Embryonic lethal at 10.5 dpc (157)	ND	Hypoxia (61)
	P4Ha2	*P4ha2*	$(GPP)_n$ single chain	Normal	High myopia (158)	Hypoxia (61)
	P4Ha3	*P4ha3*	$(GPP)_n$ single chain	ND	ND	ND
Complex with P4Ha	PDI	*P4hb*	ND	Increased ER stress in PDI-mutated cells	Cole-Carpenter syndrome (159) Amyotrophic lateral sclerosis	Hypoxia (61)
Prolyl 3-hydroxylase	P3H1	*P3h1*	Prolyl 4-hydroxylated collagen (37)	Abnormalities in collagen-rich tissues such as bones, tendons, and skin (40)	OI (40)	Hypoxia (61)
	P3H2	*P3h2*	ND	Embryonic lethal at 8.5 dpc (35)	High myopia (160)	ND
Complex with LH	P3H3	*P3h3*	ND	Collagen lysine underhydroxylation and cross-linking abnormality (50)	ND	ND
	Sc65	*P3h4*	ND	Collagen lysine underhydroxylation and cross-linking abnormality (49)	ND	ND
	CRTAP	*Crtap*	ND	Severe osteoporosis and decreased osteoid production Decreased prolyl 3-hydroxylation (38)	OI (161)	ND
LH	LH1	*Plod1*	$(Ile\text{-}Lys\text{-}Gly)_3$ (45)	Abnormal morphology of collagen fibrils in aorta and skin (162)	Ehlers-Danlos syndrome (163)	Hypoxia (61)
	LH2	*Plod2*	$(Ile\text{-}Lys\text{-}Gly)_3$ (45)	ND	Bruck syndrome (164)	Hypoxia (61)
	LH3	*Plod3*	$(Ile\text{-}Lys\text{-}Gly)_3$ (45)	Embryonic lethal at 9.5 dpc Defects in the structure of the basement membrane and in collagen fibril organization in newborn skin and lung (51)	Connective tissue disorder (165)	ND
	GLT25D1/2	*Colgalt1/2*	Deglycosylated collagen (166)	Bent wrists, eye defects, skeletal defects, and variable incidence of cleft palate and exencephaly (53)	ND	ND

(*Continued*)

Table 1 (*Continued*)

Role	Protein	Gene	Typical substrate	Phenotypes of KO mice and cells	Human disease caused by mutation[a]	Collagen-related disease conditions associated with upregulation
Peptidyl-prolyl isomerase	Cyclophilin B	*Ppib*	Succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (167)	Skeletal abnormalities including decreased mineralization and abnormal shape of calvaria, shortened limbs, and a deformed and flared rib cage Delayed procollagen secretion in KO cells (168)	OI (41)	ND
	FKBP22	*Fkbp14*	Succinyl-Ala-Ala-Hyp-Phe-*p*-nitroanilide (167)	ND	Ehlers-Danlos syndrome (62)	ND
	FKBP65	*Fkbp10*	Succinyl-Ala-Ala-Hyp-Phe-*p*-nitroanilide (167)	Died after birth Embryos present with growth delay and tissue fragility (169)	OI (170) Bruck syndrome (171)	ND
Molecular chaperone	Hsp47	*SerpinH1*	Triple-helical collagen	Embryonic lethal at 10.5 dpc (84) Delayed procollagen secretion, UPR induction	OI (86)	Fibrosis [Hsp47 is a druggable target for treating fibrosis in a Phase II study using Hsp47 siRNA (89)]
Cargo receptor at ER exit site	TANGO1	*Mia3*	Specific to collagen type VII	Short-limbed dwarfism and died at birth (107) Delayed procollagen secretion and UPR induction	Severe dentinogenesis imperfecta, short stature, various skeletal abnormalities (172)	Circadian rhythm
Cargo receptor with TANGO1	cTAGE5	*Mia2*	None	Severe fatty liver and hypolipemia in hepatocyte-specific KO mice (173)	Lower circulating VLDL, LDL, HDL, and triglycerides (174)	ND
Component of COPII	Sec24D	*Sec24d*	None	Embryonic lethal at 10.5 dpc (175)	OI (176)	ND

[a]From the Human Gene Mutation Database (**http://www.hgmd.cf.ac.uk**).

Abbreviations: CRTAP, cartilage-associated protein; dpc, days post coitum; ER, endoplasmic reticulum; FKBP, FK506-binding protein; GLT25D, glycosyltransferase 25 domain containing; HDL, high-density lipoprotein; Hsp47, heat shock protein 47; KO, knockout; LDL, low-density lipoprotein; LH, lysyl hydroxylase; ND, not determined; OI, osteogenesis imperfecta; PDI, protein disulfide isomerase; Sc65, synaptonemal complex 65; TANGO1, transport and Golgi organization 1; UPR, unfolded protein response; VLDL, very-low-density lipoprotein.

formation of 4-hydroxyproline by hydroxylating proline in the Gly-Xaa-Pro motif in single-chain procollagen. The hydroxylation reaction requires ferrous ion (Fe^{2+}), 2-oxoglutarate, molecular oxygen, and ascorbic acid (vitamin C) (29). P4Hs are localized in the ER lumen and form tetramers with 2 α subunits and 2 β subunits; the β subunit has an enzyme activity and is also known as protein disulfide isomerase (PDI) (30). Before triple-helix formation, the P4H–PDI complex associates with single-chain procollagen to hydroxylate proline at the Yaa position and thus prevents interactions between the single chains prior to trimer formation at the C propeptide end, enabling the formation of a correct, stable triple helix (31). Three P4H isoforms (P4Ha1, P4Ha2, and P4Ha3) have been identified in mammalian cells (32). P4Ha1 is the major isoform in most cell types and tissues and accounts for the majority of P4H activity.

Almost all proline residues in the Yaa position of Gly-Xaa-Yaa repeats in procollagen are prolyl 4-hydroxylated (33). 96% and 99% of prolines in the Yaa position are prolyl 4-hydroxylated in bovine type I and type V collagen, respectively (34). Other modifications at some specific sites have different roles in procollagen maturation**.** For example, prolyl 3-hydroxylation of procollagen is involved in the interaction between mature collagen and collagen-binding proteins after secretion from the cell (35). Lysyl hydroxylation of procollagen is involved in the crosslinking of mature collagen, which is mediated by hydroxyl lysine and lysyl oxidases (36).

3.2. The Prolyl 3-Hydroxylase 1–Cartilage-Associated Protein–Cyclophilin B Complex

P3Hs generate 3-hydroxyproline residues in the Gly-Pro-(4-Hyp) motif at specific sites on procollagen. P3H1 forms a complex with cartilage-associated protein (CRTAP) and the PPIase CypB in a 1:1:1 ratio and hydroxylates Pro986 in type I collagen in the ER (37, 38). Knockdown of CRTAP or CypB caused a reduction in P3H1 protein levels, suggesting the importance of this complex formation for P3H1 stability (39). Knockdown of P3H1 did not affect CypB levels; however, interactions between CypB and collagen were reduced (39), indicating that P3H1 is responsible for binding of the complex to procollagen. The absence of any component of the complex leads to a reduction in Pro986 hydroxylation and causes OI (38, 40, 41). In mice synthesizing a catalytically inactive P3H1 variant, the collagen fibrils generated therein are more homogeneous and smaller in diameter than those from wild type mice (40). The role of hydroxylated Pro986 in collagen fibrogenesis remains unclear.

P3H2 hydroxylates Pro707 in procollagen type I, as well as many proline residues in procollagen type IV (42). *P3H2* KO mice die before 8.5 dpc (35). The lethal phenotype can be rescued by producing double variants of P3H2 and maternal platelet-specific glycoprotein VI (GPVI). GPVI binds to the $(Gly\text{-}Pro\text{-}Hyp)_n$ repeat and initiates platelet aggregation upon blood exposure during injury. KO and double-KO experiments suggest that the prolyl 3-hydroxylation of type IV collagen is required for the prevention of an aberrant interaction with GPVI, which would otherwise result in platelet aggregation, thrombosis of maternal blood, and embryo death. Hence, 3-hydroxylation of type IV collagen is indispensable for embryonic development in mice (35).

3.3. The Lysyl Hydroxylase 2 Dimer–Heat Shock Protein 47–FK506-Binding Protein 65 Complex

LH catalyzes the formation of hydroxylysine (Hyl) in -X-Lys-Gly- sequences in a reaction that requires Fe^{2+}, 2-oxoglutarate, molecular oxygen, and ascorbate. In both N- and C-terminal telopeptides of fibrillar collagen, -X-Lys-Ala- and -X-Lys-Ser- sequences are also hydroxylated (43, 44). Following telopeptide lysine hydroxylation by LH2, intermolecular covalent crosslinks between

collagen telopeptides and helical domains are formed, increasing collagen stability (45). FKBP65, a 65-kDa FK506-binding protein encoded by the *Fkbp10* gene, is an ER-resident PPIase that forms a complex with LH2 (46). FKBP65 is involved in collagen crosslinking by specifically mediating the dimerization of LH2, which is required for LH2 activity. By using a proximity-ligation assay, the interaction between heat shock protein 47 (Hsp47) and FKBP65 was shown to be significantly reduced in cells expressing an OI-associated variant (M227T) of Hsp47, in which Hsp47 was destabilized and mislocalized (47). In vitro, purified FKBP65 preferentially interacts with Hsp47 rather than with type I collagen (48). FKBP65, LH2, and Hsp47 could therefore work together during procollagen maturation to contribute to the molecular stability and posttranslational modification of type I procollagen.

3.4. The Lysyl Hydroxylase 1–Prolyl 3-Hydroxylase 3–Synaptonemal Complex 65–Cyclophilin B Complex

LH1 hydroxylates procollagen type I at K87 and K930. Synaptonemal complex 65 (Sc65), a nonenzymatic member of the Leprecan family, which includes CRTAP, forms a complex with LH1, P3H3, and CypB (49, 50). In Sc65 KO cells, protein levels of LH1 and P3H3 were decreased, suggesting that complex formation is required for LH1 and P3H3 stability. The importance of complex formation for the stability of LH and prolyl hydroxylase appears to be common to enzymes modifying procollagen. Loss of Sc65 caused a reduction in lysine hydroxylation at K87 and K930 in procollagen type I and altered crosslinking, resulting in connective tissue defects, including low bone mass and skin fragility (49, 50).

3.5. Lysyl Hydroxylase 3 Dimer and Glycosyltransferase 25 Domain Containing 1/2

LHs hydroxylate specific lysine residues on procollagens, and the Hyl residues are further modified by LH3 (51, 52) and glycosyltransferase 25 domain containing 1 (GLT25D1) or 2 (GLT25D2) (53). LH3 has both LH and glycosyltransferase activity. Galactose can be attached to the 5-hydroxyl group of Hyl to form galactosyl-Hyl (Gal-Hyl), and glucose can be added to Gal-Hyl to form glucosylgalactosyl-Hyl (GlcGal-Hyl). Based on the crystal structure of LH3, the enzyme is a dimer during the hydroxylation reaction (54). Both LH3 and GLT25D1/2 knockdown decrease Gal-Hyl and GlcGal-Hyl levels (55). Recently, VIPAR (vacuolar protein sorting–associated protein 33B–interacting protein) was found to interact with LH3 and mediate LH3 trafficking to the post–Golgi apparatus (56). This VIPAR-dependent sorting was essential for the modification of lysine in multiple collagen types, suggesting that the location where LH3 acts and the significance of post-Golgi trafficking should be further studied.

Hypoxia, i.e., low-oxygen conditions, regulates the posttranslational modification of collagen and contributes to bone development and the pathogenesis of cancer (57–59). Hypoxia activates hypoxia-inducible transcription factor (HIF-1α). Because almost all prolyl-hydroxylase, LH, and lysyl oxidase genes are the targets of HIF-1α, hypoxic conditions, including those in hypoxic chondrocytes and some tumor cells, induce the expression of these procollagen-modifying enzymes. This results in increased collagen crosslinking and the accumulation of collagenous matrix because of increased collagen resistance to MMPs. This process enhances breast cancer metastasis (60, 61).

Of the PPIase families present in the ER, gene mutations of CypB, FKBP22, and FKBP65 are associated with clear collagen-related phenotypes (**Table 1**). CypB and FKBP65 form a complex with LHs. Mutation of the *FKBP22* (*FKBP14*) gene causes Ehlers–Danlos syndrome (62); however,

the underlying mechanism is unclear. The requirement of PPIase activity for collagen folding has not yet been demonstrated in vivo.

4. CHAIN ASSEMBLY AND TRIMER FORMATION

The Gly-Xaa-Yaa repeats in the collagen peptide form trimers and triple-helical structures below the melting temperature in vitro (**Figure 2*a***) (63). However, in cells, the Gly-Xaa-Yaa repeats from different collagen types (e.g., collagen types I and III) do not form heterotypic triple helices. This highlights the importance of C propeptides in triple-helix formation in vivo (**Figure 2*b***).

In the cell, association and trimer formation at the trimerization region (such as the C propeptide of collagen types I, II, and III) are required before procollagen triple-helix formation can occur. Of the 28 types of collagen, 21, including types II, III, VII, and X, are homotrimeric; however, the other seven types form heterotrimers, e.g., $(\alpha1)_2(\alpha2)$ in type I collagen (5). Notably, the α1 chain can form a trimer, while the α2 chain cannot. Further, the combination of two α2 chains and one α1 chain is not allowed in cells. In general, C propeptides facilitate the formation of a heterotrimer, and this composition is more thermally stable than a homotrimer.

In some human cancer cell lines, *col1a2* mRNA levels are reduced because of increased DNA methylation near the start codon of the *col1a2* gene (64). This results in an imbalance between levels of col1α1 and col1α2 and the production of col1α1 homotrimers. Intriguingly, col1α1 homotrimers are resistant to cleavage by MMPs because the enzymes specifically recognize the $(\alpha1)_2(\alpha2)$ heterotrimer. This suggests that invasive cancer cells may use homotrimers to build an MMP-resistant invasion area (65). A C-propeptide mutation in col1α2 has also been reported to cause OI by failing to form heteromeric triple helices with col1α1 (66). Hence, the col1α1 and col1α2 heterotrimer is critical for the turnover and quality of type I collagen through its interaction with collagen-binding proteins in the ECM.

The mechanism of heterotrimer formation was a longstanding enigma in the field. However, crystal structures of C propeptide were recently determined and provided critical evidence for understanding the trimerization mechanism (**Figure 3*a***) (67, 68). The crystal structures revealed a circularly symmetrical structure of the col1α1 homotrimer, with each chain binding one calcium ion near the interaction interface. Further, several salt bridges are formed around the calcium ions and water molecules (**Figure 3*b***). Col1α1 has eight cysteines, C1–C8, numbered from the N to the C terminus. C1–C4, C5–C8, and C6–C7 form intrachain disulfide bonds visible in the crystal structure (**Figure 3*a***). However, the C3 cysteine residue forms an interchain disulfide bond with the C2 cysteine in another chain. Importantly, col1α2 lacks the C2 cysteine, with a serine in this position; hence, col1α2 cannot form a homotrimer. $\text{Col1}\alpha2^{\text{S2C}}$ variants can form homotrimers via disulfide bonds (69). One calcium ion near the C3 cysteine residue plays a critical role in the salt-bridge network at the interface of two α1 chains (**Figure 3*c***), forming noncovalent bonds with the two chains. The $\text{col1}\alpha1^{\text{C2S}}$ variant and wild type col1α2 cannot form the disulfide bonds that mediate homotrimer formation; however, they can form a homotrimer via noncovalent bonding that depends on calcium ions, as confirmed by sedimentation equilibrium experiments (69).

Chain selectivity is explained by short, discontinuous sequences in each polypeptide chain called chain-recognition sequences (CRSs) (70). Each chain contains a distinct CRS, which ensures a collagen type–specific association. Based on the crystal structure, these CRSs are located at the interface of two C propeptides (**Figure 3*b***). Calcium ions organize the noncovalent bonds, including the salt bridges, at the interface characterized by the CRSs.

The presence or absence of a single cysteine in the C-propeptide domain in type I collagen is a key factor governing the ability of a specific collagen polypeptide to form stable homotrimers. Noncovalent or covalent heterodimers of col1α1 and col1α2 and homodimers of col1α1 form

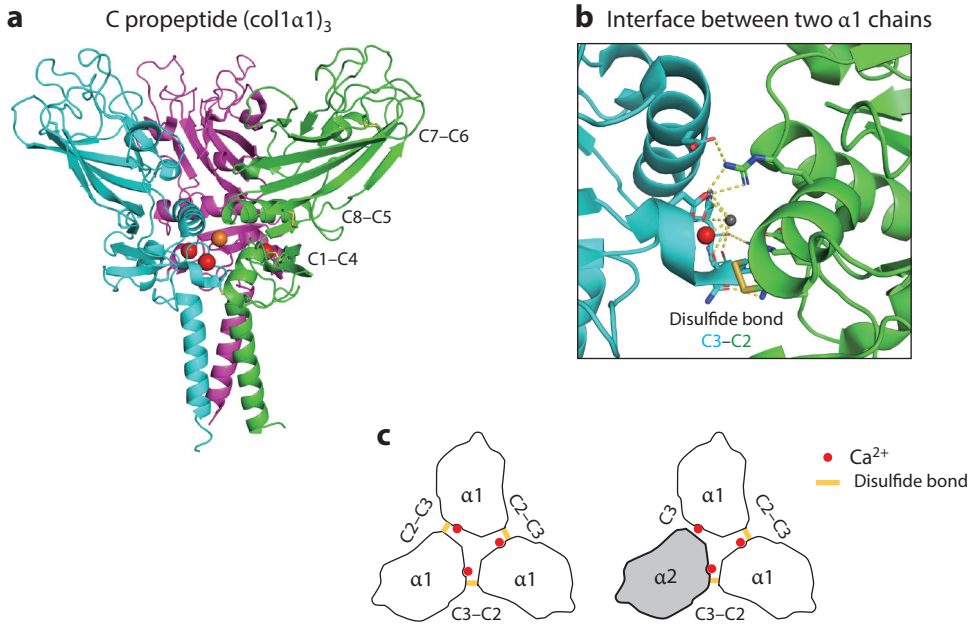


Figure 3

Trimer formation by the collagen C propeptide. (*a*) Crystal structure of the C propeptide homotrimer (col1α1) (Protein Data Bank identifier: 5K31). Each chain is shown in a different color, with the bound Ca^{2+} ions shown as red spheres and the Cl^- ion shown as an orange sphere. Disulfide bonds are shown in yellow. Cysteine residues are numbered from the N terminus, C1 to C8. C1–C4, C8–C5, and C7–C6 form intramolecular disulfide bonds. The C2 and C3 cysteines form intermolecular disulfide bonds between different chains. Col1α2 lacks the C2 cysteine. (*b*) The interaction interface between two col1α1 chains. A salt-bridge network (*dotted line*) mediates the interaction between the two chains. Ca^{2+} ions and water are critical for salt-bridge formation, affecting the formation of the intermolecular disulfide bond. (*c*) Top views of the col1α1 homotrimer and a heterotrimer formed from two col1α1 and one col1α2 chains. Col1α2, Ca^{2+}, and the disulfide bonds are shown in gray, red, and yellow, respectively.

during the trimerization process. However, it is not known whether collagen polypeptides are sufficient for heterotrimer formation alone or whether some ER oxidoreductase or chaperone aids the trimerization process.

5. THE ENDOPLASMIC RETICULUM–RESIDENT MOLECULAR CHAPERONE HEAT SHOCK PROTEIN 47 IN COLLAGEN FOLDING

While the Gly-Xaa-Yaa repeats and prolyl hydroxylation are essential for the formation of the triple-helical structure of collagen (**Figure 2*d***), temperature is another fundamental factor determining the triple-helical structure. In vivo, normal body temperature is one of the absolute factors required for normal development from an embryo to an adult (71). As type I collagen is thermally unstable even at body temperature (9), at least in mammals, correct folding of procollagen requires the presence of chaperone-like cofactor(s).

5.1. Heat Shock Protein 47 as a Collagen-Specific Molecular Chaperone

Hsp47 is an ER-resident molecular chaperone that is essential for the correct folding of procollagen in vertebrate cells. Hsp47 is encoded by the *SerpinH1* gene and belongs to the serine

protease inhibitor (serpin) superfamily, but it does not inhibit the activity of serine proteases (72). Hsp47 binds the Gly-Xaa-Arg motif of the triple-helical procollagen in the ER via hydrophobic and hydrophilic interactions and prevents its local unfolding and aggregate formation, effectively chaperoning the formation of the triple helix (72).

Hsp47 transiently associates with triple-helical procollagen in the ER in a pH-dependent manner, dissociating from procollagen below pH 6.3. It therefore dissociates from procollagen in the *cis*-Golgi or ER–Golgi intermediate compartment (ERGIC) at relatively low pHs. After dissociating from procollagen in the *cis*-Golgi or ERGIC, Hsp47 returns to the ER by binding to the KDEL receptor through its ER retention sequence (73).

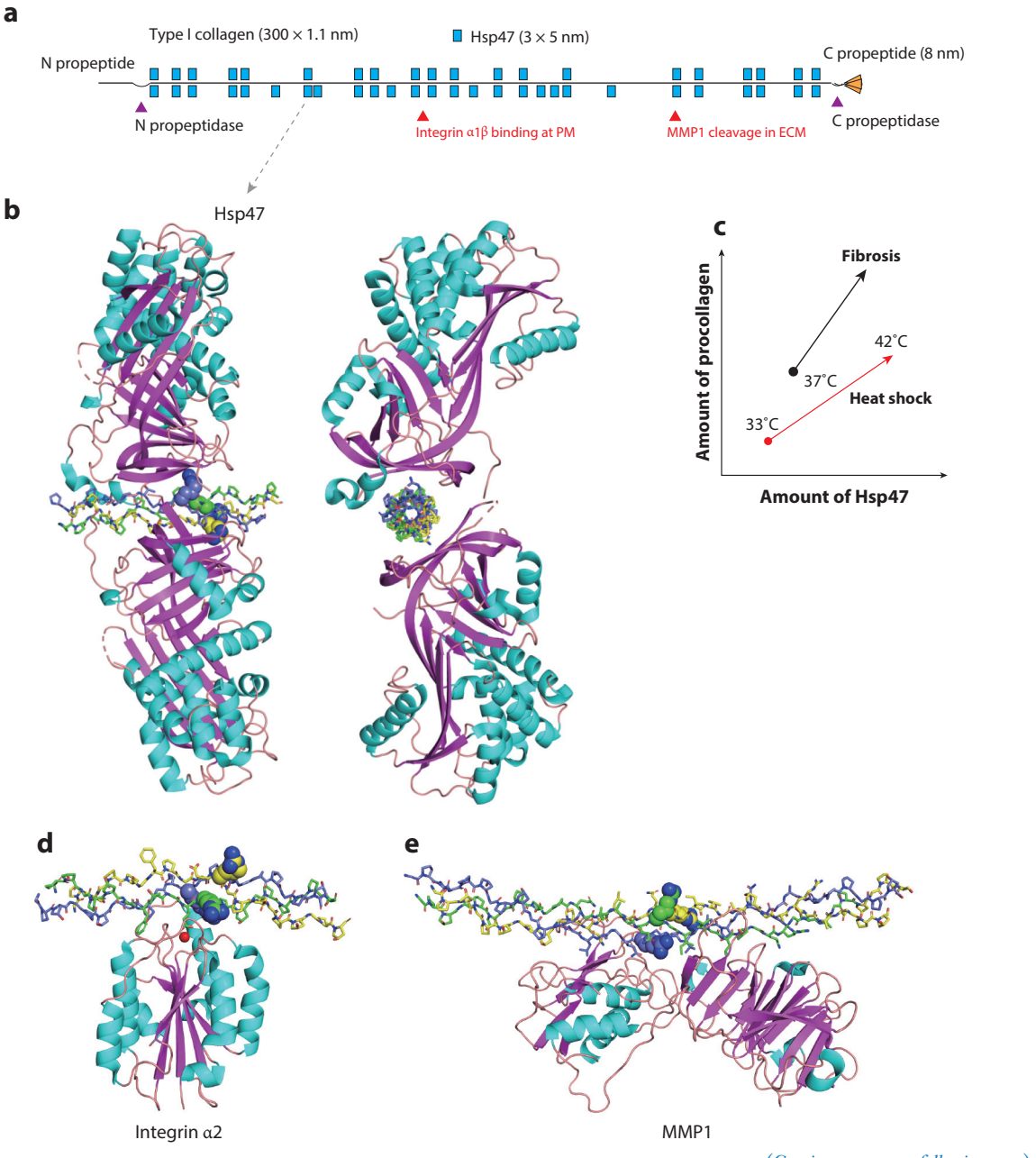
In vitro, purified recombinant Hsp47 directly binds triple-helical collagen with a high association and dissociation rate (74). While many enzymes responsible for posttranslational modification of procollagen bind the monomer form of procollagen, Hsp47 barely binds nontriple-helical procollagen. The crystal structure of the Hsp47-collagen complex revealed that Hsp47 forms a salt bridge with an arginine residue at the Yaa^{0} position in the Gly-Xaa-Yaa^{0} repeat within the triple-helical form of collagen, as well as the amino acid in the Yaa^{-3} position in the sequence Yaa^{-3}-Gly-Xaa-Arg (75, 76) (**Figure 4*b***). The residue at the Yaa^{-3} position changes the affinity of Hsp47 for collagen. When lysine, arginine, glutamine, glutamic acid, or aspartic acid is located at the Yaa^{-3} position, Hsp47 does not bind collagen (77). The crystal structure of Hsp47 revealed that collagen in its triple-helical form is bracketed by two Hsp47 molecules that bind to the same position of the collagen peptide from different angles, suggesting that Hsp47 stabilizes procollagen during formation of its triple helix (78). Hsp47 undergoes no significant conformational changes upon collagen binding. It is important to note that purified Hsp47 does not affect the melting temperature of collagen in vitro (79). However, purified Hsp47 prevents collagen fibril formation driven by the interaction between the collagen molecules (80).

In *Hsp47* KO cells, the secretion of procollagen is greatly delayed relative to wild type cells, resulting in the accumulation of procollagen in the ER. Fibrils of type I collagen produced in *Hsp47* KO cells are abnormally thin and frequently branched, and the N propeptide of secreted collagen is not processed correctly, even in the ECM (81). These findings suggest that procollagen is not correctly folded into the triple-helical form in the ER in the absence of Hsp47. When *Hsp47* KO cells are transfected with *Hsp47* wild type cDNA, collagen accumulation in the ECM is recovered; however, an Hsp47 variant Y365A, which lacks the ability to bind procollagen, fails to recover collagen accumulation, suggesting that the interaction of Hsp47 with collagen is essential for collagen maturation (82).

Type I collagen has a rod- or needle-like structure with an approximately 300-nm triple-helical region (83), an approximately 8-nm C-propeptide region (68), and an N-propeptide region of unknown size (**Figure 4*a***). Based on the crystal structure, the Hsp47–collagen complex is formed by two Hsp47 molecules that attach in a head-to-head fashion to two strands of one triple-helical collagen molecule (**Figure 4*b***). Hsp47 is engaged in hydrophobic interactions with triple-helical collagen such that a collagen molecule appears to be covered by Hsp47 protein. If the molecular size of Hsp47 is 3×5 nm (76), there are 46 or 47 possible binding sites for Hsp47 on type I procollagen, as shown in **Figure 4*a***. At present, it is not known how many Hsp47 molecules simultaneously interact with a procollagen molecule in vivo.

The correlated expression of Hsp47 with procollagen and the amount of Hsp47 bound to procollagen are important for Hsp47 to function properly as a chaperone for collagen during its synthesis in the cell. *Hsp47* KO mice are embryonic lethal beyond 11.5 dpc due to the disruption of the basement membrane caused by their abnormal synthesis of type IV collagen (84). In contrast, heterozygous *Hsp47* KO mice appear phenotypically normal, implying that half the normal amount of Hsp47 might be sufficient for the efficient production of collagen. The binding of

Hsp47 to collagens was visualized by transmission electron microscopy (78). At a low concentration, Hsp47 appeared to preferentially bind the N-terminal region. At high Hsp47 concentrations, the most frequently detected number of Hsp47 particles bound to a single procollagen I molecule was eight. However, the stoichiometry of Hsp47 to procollagen in vivo has not been reported so far.



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

The collagen chaperone Hsp47 interacts with procollagen. (*a*) Putative Hsp47-binding sites on procollagen type I. The molecular sizes determined for procollagen type I (300 × 1.1 nm), Hsp47 (3 × 5 nm), and C propeptide (8 nm) are shown. Hsp47 binds the Yaa^{-3}-Gly-Xaa-Arg sequence in triple-helical collagen. The Yaa^{-3} residue affects the affinity of Hsp47 for collagen. There are 46 or 47 putative Hsp47-binding sites on procollagen type I (*blue boxes*). (*b*, *left*) Crystal structure of the Hsp47–collagen complex (PDB ID: 3ZHA). Two Hsp47 molecules interact with the triple-helical collagen peptide (PPGPPGPTGPRGPPGPP × 3) in a head-to-head fashion. The arginine residues (*sphere model*) form a salt bridge with Hsp47. (*Right*) Rotated view of the structure. (*c*) The relationship between the amount of procollagen and the amount of Hsp47. The collagen and Hsp47 levels are correlated during heat shock and in fibrotic disease. (*d*) Structural view of the interaction of integrin α2 with the collagen peptide (PDB ID: 1DZ1). The arginine residues (*sphere model*) form a salt bridge with integrin. (*e*) Structural view of the interaction of MMP1 with the collagen peptide (PDB ID: 4AUO). Abbreviations: ECM, extracellular matrix; Hsp47, heat shock protein 47; MMP1, matrix metalloproteinase 1; PDB ID, Protein Data Bank identifier; PM, plasma membrane.

Hsp47 shares its binding site (Gly-Xaa-Arg) with the integrin α2β1 binding site (Gly-Phe-Hyp-Gly-Glu-Arg) on type I collagen, and the correct triple-helical structure of this site is essential for integrin signaling (**Figure 4*d***) (23). *Hsp47* knockdown affects integrin-mediated PI3K/AKT/IκB signaling and results in decreased procollagen mRNA levels (85). Hsp47 also shares its binding site with the collagenase MMP binding site (Gly-Ile-Ala-Gly-Gln-Arg-Gly-Val-Val-Gly-Leu-Pro-Gly) on type I collagen (**Figure 4*e***) (22). In cells from OI patients with mutations in the *Hsp47* gene, secreted type I collagen is cleaved near the collagenase site in the triple-helical domain, suggesting that Hsp47 is important for triple-helix stability and the turnover of collagen by collagenase (86).

Increased Hsp47 expression is associated with collagen-related disorders (87), such as fibrotic diseases, which are characterized by abnormal accumulation of ECM components, the major component of which is collagen (**Figure 4*c***). Following the onset of chronic inflammatory events, fibrosis proceeds via the marked induction of type I collagen and Hsp47 in parallel. Knockdown of *Hsp47* using siRNA reduces collagen accumulation and suppresses fibrosis in a mouse model, showing that Hsp47 is a promising molecular target for the treatment of fibrosis (88, 89). Regulation of collagen production by Hsp47 in fibrosis shows that there is a lower limit of Hsp47 required for collagen synthesis.

5.2. Regulation of *Hsp47* Expression

Basal expression of *Hsp47* requires an Sp-1-binding site 280 bp upstream of the transcription initiation site. Two other domains in the first and second introns regulate tissue-specific expression (90). These sequences are enhancers that act as binding motifs for Sp2/Sp3 and Kruppel-like factor (KLF)-6/Zf9 (91). Among the KLF family of proteins, Zf9 activates the expression of type I collagen and TGF-β, which are both associated with the progression of hepatic fibrosis. The Sp-1-binding sites are also important for the expression of type I collagen. These common enhancers for transcription may be responsible for the coordinated expression of type I collagen and Hsp47 in various pathologies.

Hsp47 can regulate the microenvironment of cancer cells by affecting collagen accumulation in the ECM. The transcripts of Hsp47 and collagen contain a microRNA29 (miR-29)-binding site. miR-29 levels are inversely correlated with the expression of Hsp47 in human cancer tissues. Simultaneous downregulation of miR-29 and upregulation of Hsp47 have been reported in patients with pancreatic, gastric, and cervical cancers (92, 93). The miR-29 family plays an important role in the regulation of ECM genes, including many types of collagen, suggesting that miR-29 significantly inhibits cancer cell migration and invasion. The introduction of miR-29 or the silencing of *Hsp47* in breast cancer cells suppresses malignant phenotypes by reducing collagen deposition (94).

Under thermal stress, expression of *Hsp47* is transcriptionally induced (95). Many ER-resident chaperones, including BiP and Grp94, are induced by the accumulation of misfolded proteins in the ER as part of the unfolded protein response (UPR) (96, 97). Hsp47, however, is not induced by the UPR and is the only heat shock protein among the mammalian ER-resident stress proteins (98). Upon heat shock, heat shock factor 1 (HSF-1) binds a heat shock element located 180 bp upstream from the transcription initiation site of *Hsp47* and activates *Hsp47* transcription (99). Other ER-resident stress proteins, including BiP and Grp94, do not contain heat shock elements (100). While other molecular chaperones, such as Hsp60, Hsp70, BiP, and Hsp90, exhibit broad substrate specificity, Hsp47 specifically binds to procollagen as a substrate-specific molecular chaperone (81). Even at normal body temperature (37°C), HSF-1 partially acts as a transcription factor for heat shock proteins. Knockdown of *HSF-1* results in the reduction of *Hsp47* expression by approximately 30% (101). Under thermal stress conditions, global secretion, including that of procollagen, is delayed, resulting in the accumulation of procollagen molecules in the ER (102). These conditions do not strongly induce the ER stress response, presumably because heat shock–induced Hsp47 prevents the misfolding or aggregate formation of procollagen in the ER. Interestingly, while procollagen accumulates in the ER at 37°C in *Hsp47* KO fibroblasts, the accumulation of procollagen was not observed even in *Hsp47* KO fibroblasts when cultured at 33°C (103), suggesting that Hsp47 is not always necessary for the folding of procollagen at lower temperatures. This suggests that normal body temperature is not always suitable for the correct folding of procollagen within cells, which is why procollagen folding in mammals requires Hsp47.

6. TRAFFICKING FROM THE ENDOPLASMIC RETICULUM

Procollagen folded in the ER forms rigid, rod-like structures that are approximately 300 nm in length (104). These structures are too large to enter conventional COPII-coated vesicles, which are less than 90 nm in diameter (105). This suggests that specialized factors are required for procollagen secretion from the ER. Transport and Golgi organization 1 (TANGO1) is a cargo receptor for large proteins, including procollagen and prechylomicron (106). TANGO1 is a single-pass transmembrane protein with an ER signal sequence, a Src homology 3 (SH3) domain, a coiled-coil domain, and a proline-rich domain. In mice, *Tango1* KO results in the delayed secretion of various types of collagen (including types I, II, III, IV, and VII), leading to delayed chondrocyte and bone maturation (107).

TANGO1 recruits Sec16 and cTAGE5/Sec12 to ER exit sites (ERESs) (108, 109). Sec16 acts as a gathering site for Sar1 and the inner coat protein Sec23/Sec24 to form secretion carriers. TANGO1 forms macromolecular complexes with Sec16, cTAGE5, and Sec12 at ERESs, and the complex regulates the Sar1 GTPase cycle to accomplish large cargo secretion (110). Accumulation of Sec12, a guanine-nucleotide exchange factor, at ERESs activates Sar1 at the ERES, which is required for collagen export.

SH3 domains are protein modules mediating protein–protein interactions related to cell proliferation, migration, and cytoskeletal modifications (111). Inside the ER, the SH3 domain of TANGO1 interacts with Hsp47, indicating that Hsp47 could be a guide molecule that directs collagen to special vesicles by interacting with TANGO1 (112). Purified Hsp47 was shown to directly bind the recombinant SH3 domain of TANGO1 in vitro using surface plasmon resonance. The binding of Hsp47 to type I collagen did not compete with the binding of the SH3 domain to Hsp47, suggesting that Hsp47 binds to the SH3 domain and collagen at distinct sites. Thus, Hsp47 was assumed to function as an anchor between the SH3 domain of TANGO1 and collagen and to recruit procollagen to the ERES (113). This finding may answer the important

question of how TANGO1 recognizes different types of collagen. Additional studies are still required to reveal the catch-and-release mechanisms of Hsp47, procollagen, and TANGO1 at ERESs because, while TANGO1 does not enter large COPII vesicles, Hsp47 and procollagen are packed into these vesicles. It would be intriguing to mechanistically investigate how the molecular pair of TANGO1 and Hsp47 is involved in the quality control of procollagen at ERESs, which is described in the next section.

TANGO1 organizes ERESs, together with Sec16, cTAGE5, and Sec12, at the cytosolic side of the ER and interacts with Hsp47 and procollagen at the ER luminal side. Stimulated emission depletion microscopy revealed that TANGO1 forms a ring with a diameter of 200 nm (114). TANGO1 ring assembly is mediated by TANGO1 self-association and cTAGE5 interaction. After assembly, the TANGO1 ring recruits post-ER membranes (from the ERGIC and Golgi) that fuse at the ERES to create an export route for procollagen (115, 116). TANGO1 has also been reported to make a tubular tunnel between the ER and Golgi apparatus, through which triple-helical procollagen is transported from the ER to the Golgi (116, 117). Approximately 172,000 molecules of TANGO1 and 250–500 ERESs are present in a cell, i.e., 350–700 TANGO1 molecules per ERES (117, 118). Based on the fluorescent signal of GFP-procollagen in live-cell imaging experiments, at least ten procollagen molecules gather at a single ERES for export from the ER (119).

Recently, procollagen secretion has been shown to be regulated by the circadian rhythm (120). Interestingly, in addition to the circadian changes in mRNA expression of procollagen and *TANGO1* that occur in parallel, the amount of TANGO1 protein also oscillates with the circadian rhythm. Given the half-lives of ER-resident proteins, unidentified degradation mechanisms may regulate the circadian changes in TANGO1 protein levels. TANGO1 protein expression is important for this process, as it is the rate-determining step of procollagen secretion in vivo.

7. QUALITY CONTROL OF PROCOLLAGEN AND COLLAGEN-FOLDING DISEASES

A portion of newly synthesized procollagen molecules in the ER is degraded in the cell before its secretion (121); approximately 10–30% of procollagen is degraded, depending on the cell type and the cellular conditions (122). It has not been clearly or quantitatively shown whether this degradation is due to misfolding or misassembling. Degradation of procollagen has been suggested to occur in the lysosome, an intracellular degradation organelle (123). A portion of the cytoplasm or an organelle is sequestered by an isolation membrane, which results in the formation of an autophagosome. A mature autophagosome is then fused with a lysosome for degradation (124). The ER is also a target of degradation via autophagy, known as ER-phagy. A portion of procollagen molecules retained in the ER is degraded by ER-phagy (125).

Cells use two strategies to prevent the adverse effects of misfolded protein accumulation: the ubiquitin–proteasome system and the autophagy–lysosome system (126). Proteins that are misfolded or aberrantly folded in the ER are degraded via the ER-associated degradation (ERAD) pathway or ER-phagy (127). ERAD recognizes terminally misfolded proteins and retrotranslocates them into the cytosol through the dislocon channel for proteasome degradation. Not all aberrant proteins in the ER are degraded by ERAD. ER-phagy can degrade the ER itself, as well as misfolded proteins that accumulate in the ER, e.g., aggregated α1-antitrypsin Z variant (ATZ) (128, 129), a dysferlin variant (130), misfolded procollagen (125), and misfolded forms of the membrane protein GnRHR (131).

Mice lacking autophagy-related gene 7 in chondrocytes accumulate type II procollagen in the ER and exhibit defective formation of the type II collagen fibrillar network in the ECM. This suggests that autophagy plays a fundamental role as a developmentally regulated process for bone

growth in chondrocytes (132). A fraction of type II procollagen is colocalized with the autophagic marker LC3, suggesting that type II procollagen is a substrate of autophagy in chondrocytes. Intriguingly, the collagen chaperone Hsp47 is excluded from autophagic vesicles containing procollagen. While many enzymes responsible for the posttranslational modification of procollagen bind the monomeric form of procollagen, Hsp47 binds procollagen only weakly in a nontriple-helical form. Hence, the above observation suggests that incorrectly folded procollagen molecules are sequestered away from the procollagen synthesis pathway in the ER. Fibroblast growth factor 18 (FGF18) induces autophagy in chondrocytes. In *Fgf18*$^{+/-}$ mice, the protein levels of PDI (an ER marker) and GOLPH3 (a Golgi marker) are not affected. However, type II procollagen accumulation in the ER is higher than that in wild type (*Fgf18*$^{+/+}$) mice, implying that incorrectly folded procollagen, but not all ER luminal proteins, is specifically degraded by autophagy.

Fibrotic diseases, including liver, heart, kidney, and idiopathic pulmonary fibrosis, are characterized by an abnormal accumulation of ECM components, the main component of which is collagen (133). During fibrosis progression, TGF-β induces the expression of type I collagen, Hsp47, and fibronectin (87). Chloroquine is an inhibitor of lysosomal function and is also used to inhibit autophagy in the treatment of cancer (134). Intracellular accumulation of procollagen is observed in the autophagosome in the presence of chloroquine. In a mouse model of bleomycin-induced lung fibrosis, metformin therapeutically alleviated fibrosis by activating autophagy in an AMPK-dependent manner (135), suggesting that autophagy regulates the turnover of procollagen. However, it is not known whether unfolded procollagen is selectively degraded or whether correctly folded procollagen is also degraded during ER turnover in fibrotic diseases caused by collagen overproduction.

Although the autophagy induced by nutrient starvation serves as the bulk degradation system for cytosolic proteins, cells can also selectively degrade autophagic substrates, including the ER and mitochondria, to maintain organelle quality (136–138). During such selective autophagy, autophagy receptors play an essential role in the recognition of the autophagic target. To date, six ER-phagy receptors have been identified in mammals: FAM134B (138), RTN3 (139), CCPG1 (140), Sec62 (141), ATL3 (142), and TEX264 (143, 144), all of which are ER membrane proteins localized in their specific subdomains, tubular ER or sheet ER. These receptors have an LC3 interacting region motif, are anchored at the growing isolation membranes, and help their sequestration into the autophagosome (145). FAM134B forms a complex with calnexin, an ER membrane lectin, and is involved in the degradation of procollagen in the ER (28). Knockdown of *FAM134B* or calnexin significantly decreases the numbers of type I procollagen–positive lysosomes, while knockdown of *RTN3* or *CCPG1* has a modest effect. The interaction between FAM134B, calnexin, and procollagen observed in the presence of bafilomycin A1, an inhibitor of autophagy flux, is reduced after treatment with castanospermine, which inhibits the interaction of procollagen with calnexin by inhibiting the *N*-glycan trimming of the C propeptide. Since procollagen is the most abundantly accumulated protein in mouse embryonic fibroblasts while autophagy is suppressed, procollagen is the main substrate of ER-phagic degradation mediated by the calnexin–FAM134B complex.

Although procollagen with bound Hsp47 is caught by TANGO1, and TANGO1 organizes a complex with cTAGE5, Sec12, Sec16, Sec23, and Sec24 at ERESs, FAM134B was recently reported to interact with Sec24C, a member of the COPII complex, at ER-phagy sites (146). In yeast, Lst1 (a Sec24C homolog) binds to the ER-phagy receptor Atg40 (a FAM134B homolog) and facilitates the packaging of ER into the autophagosome. ATZ is an aggregation-prone protein and is eliminated by ER-phagy when aggregated. ATZ aggregates accumulate in the pellet fraction after centrifugation of the cell lysate in *Atg40* mutant and *Lst1* mutant strains, suggesting that Atg40 and Lst1 are required for the clearance of aggregated proteins in the ER by ER-phagy

(146). Taken together, the combination of these components works as a sorting device for secretion versus degradation via ER-phagy; that is, Sec24–Sec23 cargo adaptors secrete correctly folded procollagen with Hsp47-TANGO1, and Sec24C–Sec23 cargo adaptors degrade aggregate-forming procollagen by ER-phagy with FAM134B–calnexin.

The mechanism by which only correctly folded procollagen is transported to the Golgi apparatus from the ER has been a long-standing enigma. In other words, the molecule(s) performing surveillance for correctly or incorrectly folded procollagens in the ER for further transport has never been elucidated. Hsp47 exclusively binds to correctly folded triple-helical procollagens in the ER but not to monomeric collagen peptides or loosened triple helices. Thus, by binding to the SH3 domain of TANGO1 at the ERES, Hsp47 is assumed to enable only correctly folded triple-helical procollagen to be transported from the ER to the Golgi apparatus. In this model, Hsp47 is able to recognize whether procollagen is correctly folded or not, and TANGO1 serves as the driving force for the transport of the Hsp47–procollagen complex. This model provides new mechanisms for the recognition, selection, and sorting of the ER cargo within the ER quality-control system, while these mechanisms should be studied in more detail in the future.

Mov13 cells cannot synthesize α1 chains of type I collagen due to a mutation in the *col1α1* gene, but α2 chains are synthesized (147). The α2 chains are transported to the Golgi apparatus and degraded in the lysosome, not by the autophagic pathway (148). When the α1 chains of type I collagen are introduced into Mov13 cells, they form triple helices with the endogenous α2 chains, and these triple helices are normally secreted into the ECM. However, when the α1 chain harboring a mutation in the C-propeptide region is introduced into Mov13 cells, it cannot make a trimer with the endogenous α2 chain, and the unassembled α1 chains are degraded by ERAD (149). When the α1 chain harboring a glycine substitution (G859R) in the triple-helix region is introduced into Mov13 cells, the α1 chains form a trimer with the endogenous α2 chains but fail to make a correct triple helix, and the resultant heterotrimers accumulate as detergent-insoluble aggregates; ultimately, they are removed by ER-phagy. Thus, procollagen molecules that cannot make a correct triple helix are degraded by three distinct quality-control mechanisms—lysosomal degradation, ERAD, or ER-phagy—according to the type of misfolding (**Figure 5**).

Mutations in the gene for type X collagen cause metaphyseal chondrodysplasia, Schmid type, a skeletal disorder characterized by abnormal bone formation, including short stature with abnormally short arms and legs and bowed legs (150). Altered type X procollagen molecules, including those with N617K substitutions, form aberrant disulfide-bonded dimers and accumulate in the ER due to misfolding (151, 152). This causes ER stress, ultimately disrupting growth-plate hypertrophic cell differentiation and bone growth (153). Carbamazepine (CBZ), a drug approved by the US Food and Drug Administration for use in epilepsy, bipolar disorder, and neuropathic pain, stimulates proteolysis of misfolded collagen X by stimulating both the autophagy and ubiquitin proteasomal degradation pathways and prevents liver fibrosis caused by the ATZ variant in a mouse model (154). In a mouse model of metaphyseal chondrodysplasia, Schmid type caused by an N617K substitution in collagen type X, CBZ reduces ER stress and improves the differentiation of hypertrophic chondrocytes, resulting in an increased bone growth rate and reduced skeletal dysplasia (155).

As described above, misfolded procollagen that accumulates in the ER induces the UPR and ER stress. Hsp47 is indispensable for the secretion of stable triple-helical collagen. In *HSP47* KO cells, a portion of misfolded procollagen molecules accumulates in the ER and forms detergent-insoluble aggregates, which induce splicing of *XBP1* and expression of *CHOP*, both markers of ER stress (125). When autophagy is inhibited, a larger amount of insoluble procollagen accumulates compared with soluble procollagen, implying that the insoluble procollagen molecules are selectively degraded by ER-phagy. Interestingly, NHK, a variant of α1 antitrypsin that acts as an

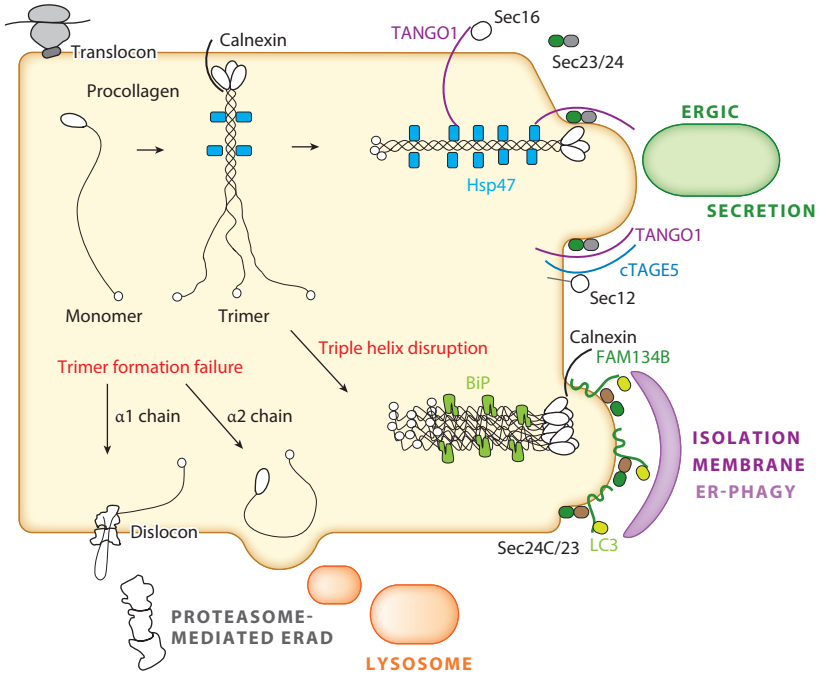


Figure 5

Cellular quality control of procollagen. Unassembled α1 and α2 are degraded by the ERAD and lysosomal pathways, respectively. After trimerization at the procollagen C propeptide, a correctly folded triple-helical procollagen is recognized and protected by the collagen-specific molecular chaperone Hsp47. The interaction between procollagen and Hsp47 is essential for collagen synthesis in vertebrate cells. At the ERES, TANGO1 organizes Sec16, cTAGE5, and Sec12 on the cytosolic side and interacts with Hsp47 and procollagen on the ER luminal side to create an export route for procollagen. Once a portion of the newly synthesized procollagen molecules in the ER is misfolded, it is sequestered from the procollagen synthetic pathway and degraded by ER-phagy. Calnexin–FAM134B, an ER-phagy receptor complex, mediates ER-phagic degradation. FAM134B interacts with Sec24C, a member of the COPII complex, at ER-phagy sites. Abbreviations: ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERES, ER exit site; Hsp47, heat shock protein; TANGO1, transport and Golgi organization 1.

ERAD model substrate, is degraded by ERAD even when autophagy is blocked, suggesting that ERAD and ER-phagy independently and also simultaneously occur at different locations in the ER. ER-phagy receptors would be a marker for the regions that are degraded by ER-phagy (156). Among ER-phagy receptors, CCPG1 and Sec62 respond to ER stress; however, FAM134B does not. It remains to be addressed which ER-phagy receptors and ER chaperones are involved in the quality control of misfolded procollagens via the ER-phagic pathway in the presence of ER stress.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research (S) (24227009) from the Japan Society for the Promotion of Science (JSPS) (to K.N.), JSPS KAKENHI grant number JP18H04002 (to K.N.), and JSPS KAKENHI grant number JP18K14393 (to S.I.). This work was partially supported by the Acceleration Transformative Research for Medical Innovation (ACT-M) from the Japan Agency for Medical Research and Development (AMED).

LITERATURE CITED

1. Neuman RE, Logan MA. 1950. The determination of collagen and elastin in tissues. *J. Biol. Chem.* 186(2):549–56
2. Niederreither K, D'Souza R, Metsäranta M, Eberspaecher H, Toman PD, et al. 1995. Coordinate patterns of expression of type I and III collagens during mouse development. *Matrix Biol.* 14(9):705–13
3. Leung AWL, Wong SYY, Chan D, Tam PPL, Cheah KSE. 2010. Loss of procollagen IIA from the anterior mesendoderm disrupts the development of mouse embryonic forebrain. *Dev. Dyn.* 239(9):2319–29
4. Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. 2004. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 131(7):1619–28
5. Ricard-Blum S. 2011. The collagen family. *Cold Spring Harb. Perspect. Biol.* 3(1):a004978
6. Bächinger HP, Bruckner P, Timpl R, Prockop DJ, Engel J. 1980. Folding mechanism of the triple helix in type-III collagen and type-III pN-collagen. Role of disulfide bridges and peptide bond isomerization. *Eur. J. Biochem.* 106(2):619–32
7. Makareeva E, Aviles NA, Leikin S. 2011. Chaperoning osteogenesis: new protein-folding disease paradigms. *Trends Cell Biol.* 21(3):168–76
8. Fidler AL, Boudko SP, Rokas A, Hudson BG. 2018. The triple helix of collagens—an ancient protein structure that enabled animal multicellularity and tissue evolution. *J. Cell Sci.* 131:jcs203950
9. Leikina E, Mertts MV, Kuznetsova N, Leikin S. 2002. Type I collagen is thermally unstable at body temperature. *PNAS* 99(3):1314–18
10. Marini JC, Forlino A, Cabral WA, Barnes AM, San Antonio JD, et al. 2007. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: Regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. *Hum. Mutat.* 28(3):209–21
11. Pepin MG, Schwarze U, Rice KM, Liu M, Leistritz D, Byers PH. 2014. Survival is affected by mutation type and molecular mechanism in vascular Ehlers-Danlos syndrome (EDS type IV). *Genet. Med.* 16(12):881–88
12. Erdmann RS, Wennemers H. 2012. Effect of sterically demanding substituents on the conformational stability of the collagen triple helix. *J. Am. Chem. Soc.* 134(41):17117–24
13. Gauba V, Hartgerink JD. 2008. Synthetic collagen heterotrimers: structural mimics of wild-type and mutant collagen type I. *J. Am. Chem. Soc.* 130(23):7509–15
14. Bella J, Eaton M, Brodsky B, Berman HM. 1994. Crystal and molecular structure of a collagen-like peptide at 1.9 Å resolution. *Science* 266(5182):75–81
15. Forlino A, Cabral WA, Barnes AM, Marini JC. 2011. New perspectives on osteogenesis imperfecta. *Nat. Rev. Endocrinol.* 7(9):540–57
16. Persikov AV, Ramshaw JA, Kirkpatrick A, Brodsky B. 2000. Amino acid propensities for the collagen triple-helix. *Biochemistry* 39(48):14960–67
17. Shoulders MD, Satyshur KA, Forest KT, Raines RT. 2010. Stereoelectronic and steric effects in side chains preorganize a protein main chain. *PNAS* 107(2):559–64
18. Shoulders MD, Raines RT. 2009. Collagen structure and stability. *Annu. Rev. Biochem.* 78:929–58
19. Maaßen A, Gebauer JM, Theres Abraham E, Grimm I, Neudörfl J-M, et al. 2020. Triple-helix-stabilizing effects in collagen model peptides containing PPII-helix-preorganized diproline modules. *Angew. Chem. Int. Ed.* 59(14):5747–55

20. Kramer RZ, Bella J, Mayville P, Brodsky B, Berman HM. 1999. Sequence dependent conformational variations of collagen triple-helical structure. *Nat. Struct. Biol.* 6(5):454–57
21. Boudko SP, Engel J, Okuyama K, Mizuno K, Bächinger HP, Schumacher MA. 2008. Crystal structure of human type III collagen Gly^{991}–Gly^{1032} cystine knot-containing peptide shows both 7/2 and 10/3 triple helical symmetries. *J. Biol. Chem.* 283(47):32580–89
22. Manka SW, Carafoli F, Visse R, Bihan D, Raynal N, et al. 2012. Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. *PNAS* 109(31):12461–66
23. Emsley J, Knight CG, Farndale RW, Barnes MJ, Liddington RC. 2000. Structural basis of collagen recognition by integrin α2β1. *Cell* 101(1):47–56
24. Dzamba BJ, Wu H, Jaenisch R, Peters DM. 1993. Fibronectin binding site in type I collagen regulates fibronectin fibril formation. *J. Cell Biol.* 121(5):1165–72
25. Zheng H, Lu C, Lan J, Fan S, Nanda V, Xu F. 2018. How electrostatic networks modulate specificity and stability of collagen. *PNAS* 115(24):6207–12
26. Gauba V, Hartgerink JD. 2007. Self-assembled heterotrimeric collagen triple helices directed through electrostatic interactions. *J. Am. Chem. Soc.* 129(9):2683–90
27. Hwang J, Huang Y, Burwell TJ, Peterson NC, Connor J, et al. 2017. In situ imaging of tissue remodeling with collagen hybridizing peptides. *ACS Nano* 11(10):9825–35
28. Forrester A, De Leonibus C, Grumati P, Fasana E, Piemontese M, et al. 2019. A selective ER-phagy exerts procollagen quality control via a Calnexin-FAM134B complex. *EMBO J.* 38:e99847
29. Rappu P, Salo AM, Myllyharju J, Heino J. 2019. Role of prolyl hydroxylation in the molecular interactions of collagens. *Essays Biochem.* 63(3):325–35
30. Berg RA, Prockop DJ. 1973. Affinity column purification of protocollagen proline hydroxylase from chick embryos and further characterization of the enzyme. *J. Biol. Chem.* 248(4):1175–82
31. Wilson R, Lees JF, Bulleid NJ. 1998. Protein disulfide isomerase acts as a molecular chaperone during the assembly of procollagen. *J. Biol. Chem.* 273(16):9637–43
32. Myllyharju J, Kivirikko KI. 2004. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet.* 20(1):33–43
33. Taga Y, Kusubata M, Ogawa-Goto K, Hattori S. 2014. Stable isotope-labeled collagen: a novel and versatile tool for quantitative collagen analyses using mass spectrometry. *J. Proteome Res.* 13(8):3671–78
34. Taga Y, Kusubata M, Mizuno K. 2020. Quantitative analysis of the positional distribution of hydroxyproline in collagenous Gly-Xaa-Yaa sequences by LC-MS with partial acid hydrolysis and precolumn derivatization. *Anal. Chem.* 92(12):8427–34
35. Pokidysheva E, Boudko S, Vranka J, Zientek K, Maddox K, et al. 2014. Biological role of prolyl 3-hydroxylation in type IV collagen. *PNAS* 111(1):161–66
36. Eyre DR, Weis MA. 2013. Bone collagen: new clues to its mineralization mechanism from recessive osteogenesis imperfecta. *Calcif. Tissue Int.* 93(4):338–47
37. Vranka JA, Sakai LY, Bächinger HP. 2004. Prolyl 3-hydroxylase 1, enzyme characterization and identification of a novel family of enzymes. *J. Biol. Chem.* 279(22):23615–21
38. Morello R, Bertin TK, Chen Y, Hicks J, Tonachini L, et al. 2006. CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell* 127(2):291–304
39. Choi JW, Sutor SL, Lindquist L, Evans GL, Madden BJ, et al. 2009. Severe osteogenesis imperfecta in cyclophilin B–deficient mice. *PLOS Genet.* 5(12):e1000750
40. Cabral WA, Chang W, Barnes AM, Weis M, Scott MA, et al. 2007. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nat. Genet.* 39(3):359–65
41. van Dijk FS, Nesbitt IM, Zwikstra EH, Nikkels PGJ, Piersma SR, et al. 2009. *PPIB* mutations cause severe osteogenesis imperfecta. *Am. J. Hum. Genet.* 85(4):521–27
42. Fernandes RJ, Farnand AW, Traeger GR, Weis MA, Eyre DR. 2011. A role for prolyl 3-hydroxylase 2 in post-translational modification of fibril-forming collagens. *J. Biol. Chem.* 286(35):30662–69
43. Kivirikko KI, Ryhänen L, Anttinen H, Bornstein P, Prockop DJ. 1973. Further hydroxylation of lysyl residues in collagen by protocollagen lysyl hydroxylase in vitro. *Biochemistry* 12(24):4966–71
44. Yamauchi M, Sricholpech M. 2012. Lysine post-translational modifications of collagen. *Essays Biochem.* 52:113–33

45. Takaluoma K, Lantto J, Myllyharju J. 2007. Lysyl hydroxylase 2 is a specific telopeptide hydroxylase, while all three isoenzymes hydroxylate collagenous sequences. *Matrix Biol*. 26(5):396–403
46. Gjaltema RAF, van der Stoel MM, Boersema M, Bank RA. 2016. Disentangling mechanisms involved in collagen pyridinoline cross-linking: The immunophilin FKBP65 is critical for dimerization of lysyl hydroxylase 2. *PNAS* 113(26):7142–47
47. Duran I, Martin JH, Weis MA, Krejci P, Konik P, et al. 2017. A chaperone complex formed by HSP47, FKBP65, and BiP modulates telopeptide lysyl hydroxylation of type I procollagen. *J. Bone Miner. Res.* 32(6):1309–19
48. Ishikawa Y, Holden P, Bächinger HP. 2017. Heat shock protein 47 and 65-kDa FK506-binding protein weakly but synergistically interact during collagen folding in the endoplasmic reticulum. *J. Biol. Chem.* 292(42):17216–24
49. Heard ME, Besio R, Weis M, Rai J, Hudson DM, et al. 2016. Sc65-null mice provide evidence for a novel endoplasmic reticulum complex regulating collagen lysyl hydroxylation. *PLOS Genet*. 12(4):e1006002
50. Hudson DM, Weis M, Rai J, Joeng KS, Dimori M, et al. 2017. P3h3-null and Sc65-null mice phenocopy the collagen lysine under-hydroxylation and cross-linking abnormality of Ehlers-Danlos syndrome type VIA. *J. Biol. Chem.* 292(9):3877–87
51. Ruotsalainen H, Sipilä L, Vapola M, Sormunen R, Salo AM, et al. 2006. Glycosylation catalyzed by lysyl hydroxylase 3 is essential for basement membranes. *J. Cell Sci.* 119(Part 4):625–35
52. Rautavuoma K, Takaluoma K, Sormunen R, Myllyharju J, Kivirikko KI, Soininen R. 2004. Premature aggregation of type IV collagen and early lethality in lysyl hydroxylase 3 null mice. *PNAS* 101(39):14120–25
53. Geister KA, Timms AE, Beier DR. 2018. Optimizing genomic methods for mapping and identification of candidate variants in ENU mutagenesis screens using inbred mice. *G3: Genes Genomes Genet.* 8(2):401–9
54. Scietti L, Chiapparino A, De Giorgi F, Fumagalli M, Khoriauli L, et al. 2018. Molecular architecture of the multifunctional collagen lysyl hydroxylase and glycosyltransferase LH3. *Nat. Commun.* 9(1):3163
55. Baumann S, Hennet T. 2016. Collagen accumulation in osteosarcoma cells lacking GLT25D1 collagen galactosyltransferase. *J. Biol. Chem.* 291(35):18514–24
56. Banushi B, Forneris F, Straatman-Iwanowska A, Strange A, Lyne A-M, et al. 2016. Regulation of post-Golgi LH3 trafficking is essential for collagen homeostasis. *Nat. Commun.* 7:12111
57. Bentovim L, Amarilio R, Zelzer E. 2013. HIF1α is a central regulator of collagen hydroxylation and secretion under hypoxia during bone development. *Development* 139(23):4473–83. Correction. 2013. *Development* 140(1):248
58. Gilkes DM, Chaturvedi P, Bajpai S, Wong CC, Wei H, et al. 2013. Collagen prolyl hydroxylases are essential for breast cancer metastasis. *Cancer Res.* 73(11):3285–96
59. Eisinger-Mathason TSK, Zhang M, Qiu Q, Skuli N, Nakazawa MS, et al. 2013. Hypoxia-dependent modification of collagen networks promotes sarcoma metastasis. *Cancer Discov*. 3(10):1190–205
60. Xiong G, Stewart RL, Chen J, Gao T, Scott TL, et al. 2018. Collagen prolyl 4-hydroxylase 1 is essential for HIF-1α stabilization and TNBC chemoresistance. *Nat. Commun.* 9(1):4456
61. Stegen S, Laperre K, Eelen G, Rinaldi G, Fraisl P, et al. 2019. HIF-1α metabolically controls collagen synthesis and modification in chondrocytes. *Nature* 565(7740):511–15
62. Baumann M, Giunta C, Krabichler B, Rüschendorf F, Zoppi N, et al. 2012. Mutations in *FKBP14* cause a variant of Ehlers-Danlos syndrome with progressive kyphoscoliosis, myopathy, and hearing loss. *Am. J. Hum. Genet.* 90(2):201–16
63. Madhan B, Xiao J, Thiagarajan G, Baum J, Brodsky B. 2008. NMR monitoring of chain-specific stability in heterotrimeric collagen peptides. *J. Am. Chem. Soc.* 130(41):13520–21
64. Sengupta PK, Smith EM, Kim K, Murnane MJ, Smith BD. 2003. DNA hypermethylation near the transcription start site of collagen α2(I) gene occurs in both cancer cell lines and primary colorectal cancers. *Cancer Res.* 63:1789–97
65. Makareeva E, Han S, Vera JC, Sackett DL, Holmbeck K, et al. 2010. Carcinomas contain a matrix metalloproteinase-resistant isoform of type I collagen exerting selective support to invasion. *Cancer Res.* 70(11):4366–74

66. Pace JM, Wiese M, Drenguis AS, Kuznetsova N, Leikin S, et al. 2008. Defective C-propeptides of the proα2(I) chain of type I procollagen impede molecular assembly and result in osteogenesis imperfecta. *J. Biol. Chem.* 283(23):16061–67
67. Bourhis J-M, Mariano N, Zhao Y, Harlos K, Exposito J-Y, et al. 2012. Structural basis of fibrillar collagen trimerization and related genetic disorders. *Nat. Struct. Mol. Biol.* 19(10):1031–36
68. Sharma U, Carrique L, Vadon-Le Goff S, Mariano N, Georges R-N, et al. 2017. Structural basis of homo- and heterotrimerization of collagen I. *Nat. Commun.* 8:14671
69. DiChiara AS, Li RC, Suen PH, Hosseini AS, Taylor RJ, et al. 2018. A cysteine-based molecular code informs collagen C-propeptide assembly. *Nat. Commun.* 9(1):4206
70. Lees JF, Tasab M, Bulleid NJ. 1997. Identification of the molecular recognition sequence which determines the type-specific assembly of procollagen. *EMBO J.* 16(5):908–16
71. Serrat MA, King D, Lovejoy CO. 2008. Temperature regulates limb length in homeotherms by directly modulating cartilage growth. *PNAS* 105(49):19348–53
72. Ito S, Nagata K. 2017. Biology of Hsp47 (Serpin H1), a collagen-specific molecular chaperone. *Semin. Cell Dev. Biol.* 62:142–51
73. 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(2):469–83
74. Natsume T, Koide T, Yokota S, Hirayoshi K, Nagata K. 1994. Interactions between collagen-binding stress protein HSP47 and collagen. Analysis of kinetic parameters by surface plasmon resonance biosensor. *J. Biol. Chem.* 269(49):31224–28
75. Koide T, Takahara Y, Asada S, Nagata K. 2002. Xaa-Arg-Gly triplets in the collagen triple helix are dominant binding sites for the molecular chaperone HSP47. *J. Biol. Chem.* 277(8):6178–82
76. Widmer C, Gebauer JM, Brunstein E, Rosenbaum S, Zaucke F, et al. 2012. Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. *PNAS* 109(33):13243–47
77. Koide T, Nishikawa Y, Asada S, Yamazaki CM, Takahara Y, et al. 2006. Specific recognition of the collagen triple helix by chaperone HSP47. II. The HSP47-binding structural motif in collagens and related proteins. *J. Biol. Chem.* 281(16):11177–85
78. Köhler A, Mörgelin M, Gebauer JM, Öcal S, Imhof T, et al. 2020. New specific HSP47 functions in collagen subfamily chaperoning. *FASEB J.* 34(9):12040–52
79. Macdonald JR, Bächinger HP. 2001. HSP47 binds cooperatively to triple helical type I collagen but has little effect on the thermal stability or rate of refolding. *J. Biol. Chem.* 276(27):25399–403
80. Thomson CA, Ananthanarayanan VS. 2000. Structure–function studies on Hsp47: pH-dependent inhibition of collagen fibril formation *in vitro*. *Biochem. J.* 349(Part 3):877–83
81. Ishida Y, Kubota H, Yamamoto A, Kitamura A, Bächinger HP, Nagata K. 2006. Type I collagen in Hsp47-null cells is aggregated in endoplasmic reticulum and deficient in N-propeptide processing and fibrillogenesis. *Mol. Biol. Cell* 17(5):2346–55
82. Ito S, Ogawa K, Takeuchi K, Takagi M, Yoshida M, et al. 2017. A small-molecule compound inhibits a collagen-specific molecular chaperone and could represent a potential remedy for fibrosis. *J. Biol. Chem.* 292(49):20076–85
83. Orgel JPRO, Irving TC, Miller A, Wess TJ. 2006. Microfibrillar structure of type I collagen in situ. *PNAS* 103(24):9001–5
84. Nagai N, Hosokawa M, Itohara S, Adachi E, Matsushita T, et al. 2000. Embryonic lethality of molecular chaperone Hsp47 knockout mice is associated with defects in collagen biosynthesis. *J. Cell Biol.* 150(6):1499–506
85. Birukawa NK, Murase K, Sato Y, Kosaka A, Yoneda A, et al. 2014. Activated hepatic stellate cells are dependent on self-collagen, cleaved by membrane type 1 matrix metalloproteinase for their growth. *J. Biol. Chem.* 289(29):20209–21
86. Christiansen HE, Schwarze U, Pyott SM, AlSwaid A, Al Balwi M, et al. 2010. Homozygosity for a missense mutation in *SERPINH1*, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am. J. Hum. Genet.* 86(3):389–98

87. Masuda H, Fukumoto M, Hirayoshi K, Nagata K. 1994. Coexpression of the collagen-binding stress protein HSP47 gene and the alpha 1(I) and alpha 1(III) collagen genes in carbon tetrachloride-induced rat liver fibrosis. *J. Clin. Investig.* 94(6):2481–88
88. Sunamoto M, Kuze K, Tsuji H, Ohishi N, Yagi K, et al. 1998. Antisense oligonucleotides against collagen-binding stress protein HSP47 suppress collagen accumulation in experimental glomerulonephritis. *Lab. Investig.* 78(8):967–72
89. Sato Y, Murase K, Kato J, Kobune M, Sato T, et al. 2008. Resolution of liver cirrhosis using vitamin A–coupled liposomes to deliver siRNA against a collagen-specific chaperone. *Nat. Biotechnol.* 26(4):431–42
90. Hirata H, Yamamura I, Yasuda K, Kobayashi A, Tada N, et al. 1999. Separate cis-acting DNA elements control cell type- and tissue-specific expression of collagen binding molecular chaperone HSP47. *J. Biol. Chem.* 274(50):35703–10
91. Yasuda K, Hirayoshi K, Hirata H, Kubota H, Hosokawa N, Nagata K. 2002. The Kruppel-like factor Zf9 and proteins in the Sp1 family regulate the expression of HSP47, a collagen-specific molecular chaperone. *J. Biol. Chem.* 277(47):44613–22
92. Yamamoto N, Kinoshita T, Nohata N, Yoshino H, Itesako T, et al. 2013. Tumor-suppressive microRNA-29a inhibits cancer cell migration and invasion via targeting HSP47 in cervical squamous cell carcinoma. *Int. J. Oncol.* 43(6):1855–63
93. Zhao D, Jiang X, Yao C, Zhang L, Liu H, et al. 2014. Heat shock protein 47 regulated by miR-29a to enhance glioma tumor growth and invasion. *J. Neurooncol.* 118(1):39–47
94. Zhu J, Xiong G, Fu H, Evers BM, Zhou BP, Xu R. 2015. Chaperone Hsp47 drives malignant growth and invasion by modulating an ECM gene network. *Cancer Res.* 75(8):1580–91
95. Nagata K, Saga S, Yamada KM. 1986. A major collagen-binding protein of chick embryo fibroblasts is a novel heat shock protein. *J. Cell Biol.* 103(1):223–29
96. Mori K. 2009. Signalling pathways in the unfolded protein response: development from yeast to mammals. *J. Biochem.* 146(6):743–50
97. Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334(6059):1081–86
98. Eletto D, Maganty A, Eletto D, Dersh D, Makarewich C, et al. 2012. Limitation of individual folding resources in the ER leads to outcomes distinct from the unfolded protein response. *J. Cell Sci.* 125(Part 20):4865–75
99. Hosokawa N, Takechi H, Yokota S, Hirayoshi K, Nagata K. 1993. Structure of the gene encoding the mouse 47-kDa heat-shock protein (HSP47). *Gene* 126(2):187–93
100. Mori K, Sant A, Kohno K, Normington K, Gething MJ, Sambrook JF. 1992. A 22 bp *cis*-acting element is necessary and sufficient for the induction of the yeast *KAR2* (BiP) gene by unfolded proteins. *EMBO J.* 11(7):2583–93
101. Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, et al. 2012. HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell* 150(3):549–62
102. Torre-Blanco A, Adachi E, Hojima Y, Wootton JA, Minor RR, Prockop DJ. 1992. Temperature-induced post-translational over-modification of type I procollagen. Effects of over-modification of the protein on the rate of cleavage by procollagen N-proteinase and on self-assembly of collagen into fibrils. *J. Biol. Chem.* 267(4):2650–55
103. Fujii KK, Taga Y, Sakai T, Ito S, Hattori S, et al. 2019. Lowering the culture temperature corrects collagen abnormalities caused by HSP47 gene knockout. *Sci. Rep.* 9(1):17433
104. Bonfanti L, Mironov AA Jr, Martínez-Menárguez JA, Martella O, Fusella A, et al. 1998. Procollagen traverses the Golgi stack without leaving the lumen of cisternae: evidence for cisternal maturation. *Cell* 95(7):993–1003
105. Spang A, Matsuoka K, Hamamoto S, Schekman R, Orci L. 1998. Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes. *PNAS* 95(19):11199–204
106. Saito K, Chen M, Bard F, Chen S, Zhou H, et al. 2009. TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. *Cell* 136(5):891–902
107. Wilson DG, Phamluong K, Li L, Sun M, Cao TC, et al. 2011. Global defects in collagen secretion in a *Mia3/TANGO1* knockout mouse. *J. Cell Biol.* 193(5):935–51

108. Saito K, Yamashiro K, Shimazu N, Tanabe T, Kontani K, Katada T. 2014. Concentration of Sec12 at ER exit sites via interaction with cTAGE5 is required for collagen export. *J. Cell Biol.* 206(6):751–62
109. Maeda M, Katada T, Saito K. 2017. TANGO1 recruits Sec16 to coordinately organize ER exit sites for efficient secretion. *J. Cell Biol.* 216(6):1731–43
110. Venditti R, Scanu T, Santoro M, Di Tullio G, Spaar A, et al. 2012. Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science* 337(6102):1668–72
111. Teyra J, Huang H, Jain S, Guan X, Dong A, et al. 2017. Comprehensive analysis of the human SH3 domain family reveals a wide variety of non-canonical specificities. *Structure* 25(10):1598–1610.e3
112. Ishikawa Y, Ito S, Nagata K, Sakai LY, Bächinger HP. 2016. Intracellular mechanisms of molecular recognition and sorting for transport of large extracellular matrix molecules. *PNAS* 113(41):E6036–44
113. Yuan L, Kenny SJ, Hemmati J, Xu K, Schekman R. 2018. TANGO1 and SEC12 are copackaged with procollagen I to facilitate the generation of large COPII carriers. *PNAS* 115(52):E12255–64
114. Raote I, Ortega Bellido M, Pirozzi M, Zhang C, Melville D, et al. 2017. TANGO1 assembles into rings around COPII coats at ER exit sites. *J. Cell Biol.* 216(4):901–9
115. Luo C, Pook E, Tang B, Zhang W, Li S, et al. 2017. Androgen inhibits key atherosclerotic processes by directly activating *ADTRP* transcription. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863(9):2319–32
116. Raote I, Ortega-Bellido M, Santos AJ, Foresti O, Zhang C, et al. 2018. TANGO1 builds a machine for collagen export by recruiting and spatially organizing COPII, tethers and membranes. *eLife* 7:e32723
117. Raote I, Ernst AM, Campelo F, Rothman JE, Pincet F, Malhotra V. 2020. TANGO1 membrane helices create a lipid diffusion barrier at curved membranes. *eLife* 9:e57822
118. Itzhak DN, Tyanova S, Cox J, Borner GH. 2016. Global, quantitative and dynamic mapping of protein subcellular localization. *eLife* 5:e16950
119. McCaughey J, Stevenson NL, Cross S, Stephens DJ. 2019. ER-to-Golgi trafficking of procollagen in the absence of large carriers. *J. Cell Biol.* 218(3):929–48
120. Chang J, Garva R, Pickard A, Yeung C-YC, Mallikarjun V, et al. 2020. Circadian control of the secretory pathway maintains collagen homeostasis. *Nat. Cell Biol.* 22(1):74–86
121. Bienkowski RS, Baum BJ, Crystal RG. 1978. Fibroblasts degrade newly synthesised collagen within the cell before secretion. *Nature* 276(5686):413–16
122. Rennard SI, Stier LE, Crystal RG. 1982. Intracellular degradation of newly synthesized collagen. *J. Investig. Dermatol.* 79(Suppl. 1):77s–82s
123. Berg RA, Schwartz ML, Crystal RG. 1980. Regulation of the production of secretory proteins: intracellular degradation of newly synthesized "defective" collagen. *PNAS* 77(8):4746–50
124. Mizushima N, Yoshimori T, Ohsumi Y. 2011. The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell Dev. Biol.* 27:107–32
125. Ishida Y, Yamamoto A, Kitamura A, Lamandé SR, Yoshimori T, et al. 2009. Autophagic elimination of misfolded procollagen aggregates in the endoplasmic reticulum as a means of cell protection. *Mol. Biol. Cell* 20(11):2744–54
126. Pohl C, Dikic I. 2019. Cellular quality control by the ubiquitin-proteasome system and autophagy. *Science* 366(6467):818–22
127. Oikonomou C, Hendershot LM. 2020. Disposing of misfolded ER proteins: a troubled substrate's way out of the ER. *Mol. Cell. Endocrinol.* 500:110630
128. Teckman JH, Perlmutter DH. 2000. Retention of mutant α_1-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279(5):G961–74
129. Kamimoto T, Shoji S, Hidvegi T, Mizushima N, Umebayashi K, et al. 2006. Intracellular inclusions containing mutant α_1-antitrypsin Z are propagated in the absence of autophagic activity. *J. Biol. Chem.* 281(7):4467–76
130. Fujita E, Kouroku Y, Isoai A, Kumagai H, Misutani A, et al. 2007. Two endoplasmic reticulum–associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). *Hum. Mol. Genet.* 16(6):618–29
131. Houck SA, Ren HY, Madden VJ, Bonner JN, Conlin MP, et al. 2014. Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. *Mol. Cell* 54(1):166–79

132. Cinque L, Forrester A, Bartolomeo R, Svelto M, Venditti R, et al. 2015. FGF signalling regulates bone growth through autophagy. *Nature* 528(7581):272–75
133. Duffield JS, Lupher M, Thannickal VJ, Wynn TA. 2013. Host responses in tissue repair and fibrosis. *Annu. Rev. Pathol. Mech. Dis.* 8:241–76
134. Maclean KH, Dorsey FC, Cleveland JL, Kastan MB. 2008. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J. Clin. Investig.* 118(1):79–88
135. Rangarajan S, Bone NB, Zmijewska AA, Jiang S, Park DW, et al. 2018. Metformin reverses established lung fibrosis in a bleomycin model. *Nat. Med.* 24(8):1121–27
136. Okamoto K, Kondo-Okamoto N, Ohsumi Y. 2009. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev. Cell* 17(1):87–97
137. Schuck S, Gallagher CM, Walter P. 2014. ER-phagy mediates selective degradation of endoplasmic reticulum independently of the core autophagy machinery. *J. Cell Sci.* 127(Part 18):4078–88
138. Khaminets A, Heinrich T, Mari M, Grumati P, Huebner AK, et al. 2015. Regulation of endoplasmic reticulum turnover by selective autophagy. *Nature* 522(7556):354–58
139. Chen R, Jin R, Wu L, Ye X, Yang Y, et al. 2011. Reticulon 3 attenuates the clearance of cytosolic prion aggregates via inhibiting autophagy. *Autophagy* 7(2):205–16
140. Smith MD, Harley ME, Kemp AJ, Wills J, Lee M, et al. 2018. CCPG1 is a non-canonical autophagy cargo receptor essential for ER-phagy and pancreatic ER proteostasis. *Dev. Cell* 44(2):217–32.e11
141. Fumagalli F, Noack J, Bergmann TJ, Cebollero E, Pisoni GB, et al. 2016. Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery. *Nat. Cell Biol.* 18(11):1173–84
142. Chen Q, Xiao Y, Chai P, Zheng P, Teng J, Chen J. 2019. ATL3 is a tubular ER-phagy receptor for GABARAP-mediated selective autophagy. *Curr. Biol.* 29(5):846–55.e6
143. Chino H, Hatta T, Natsume T, Mizushima N. 2019. Intrinsically disordered protein TEX264 mediates ER-phagy. *Mol. Cell* 74(5):909–21.e6
144. An H, Ordureau A, Paulo JA, Shoemaker CJ, Denic V, Harper JW. 2019. TEX264 is an endoplasmic reticulum-resident ATG8-interacting protein critical for ER remodeling during nutrient stress. *Mol. Cell* 74(5):891–908.e10
145. Nakatogawa H. 2020. Autophagic degradation of the endoplasmic reticulum. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 96(1):1–9
146. Cui Y, Parashar S, Zahoor M, Needham PG, Mari M, et al. 2019. A COPII subunit acts with an autophagy receptor to target endoplasmic reticulum for degradation. *Science* 365(6448):53–60
147. Schnieke A, Harbers K, Jaenisch R. 1983. Embryonic lethal mutation in mice induced by retrovirus insertion into the α1(I) collagen gene. *Nature* 304(5924):315–20
148. Gotkin MG, Ripley CR, Lamande SR, Bateman JF, Bienkowski RS. 2004. Intracellular trafficking and degradation of unassociated proα2 chains of collagen type I. *Exp. Cell Res.* 296(2):307–16
149. Fitzgerald J, Lamande SR, Bateman JF. 1999. Proteasomal degradation of unassembled mutant type I collagen pro-α1(I) chains. *J. Biol. Chem.* 274(39):27392–98
150. Warman ML, Abbott M, Apte SS, Hefferon T, McIntosh I, et al. 1993. A type X collagen mutation causes Schmid metaphyseal chondrodysplasia. *Nat. Genet.* 5(1):79–82
151. Bonaventure J, Chaminade F, Maroteaux P. 1995. Mutations in three subdomains of the carboxy-terminal region of collagen type X account for most of the Schmid metaphyseal dysplasias. *Hum. Genet.* 96(1):58–64
152. Wilson R, Freddi S, Bateman JF. 2002. Collagen X chains harboring Schmid metaphyseal chondrodysplasia NC1 domain mutations are selectively retained and degraded in stably transfected cells. *J. Biol. Chem.* 277(15):12516–24
153. Cameron TL, Bell KM, Gresshoff IL, Sampurno L, Mullan L, et al. 2015. XBP1-independent UPR pathways suppress C/EBP-β mediated chondrocyte differentiation in ER-stress related skeletal disease. *PLOS Genet.* 11(9):e1005505
154. Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, et al. 2010. An autophagy-enhancing drug promotes degradation of mutant α_1-antitrypsin Z and reduces hepatic fibrosis. *Science* 329(5988):229–32
155. Mullan LA, Mularczyk EJ, Kung LH, Forouhan M, Wragg JM, et al. 2017. Increased intracellular proteolysis reduces disease severity in an ER stress–associated dwarfism. *J. Clin. Investig.* 127(10):3861–65

156. Mochida K, Yamasaki A, Matoba K, Kirisako H, Noda NN, Nakatogawa H. 2020. Super-assembly of ER-phagy receptor Atg40 induces local ER remodeling at contacts with forming autophagosomal membranes. *Nat. Commun.* 11(1):3306
157. Holster T, Pakkanen O, Soininen R, Sormunen R, Nokelainen M, et al. 2007. Loss of assembly of the main basement membrane collagen, type IV, but not fibril-forming collagens and embryonic death in collagen prolyl 4-hydroxylase I null mice. *J. Biol. Chem.* 282(4):2512–19
158. Guo H, Tong P, Liu Y, Xia L, Wang T, et al. 2015. Mutations of *P4HA2* encoding prolyl 4-hydroxylase 2 are associated with nonsyndromic high myopia. *Genet. Med.* 17(4):300–6
159. Rauch F, Fahiminiya S, Majewski J, Carrot-Zhang J, Boudko S, et al. 2015. Cole-Carpenter syndrome is caused by a heterozygous missense mutation in *P4HB*. *Am. J. Hum. Genet.* 96(3):425–31
160. Mordechai S, Gradstein L, Pasanen A, Ofir R, El Amour K, et al. 2011. High myopia caused by a mutation in *LEPREL1*, encoding prolyl 3-hydroxylase 2. *Am. J. Hum. Genet.* 89(3):438–45
161. Barnes AM, Chang W, Morello R, Cabral WA, Weis M, et al. 2006. Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta. *N. Engl. J. Med.* 355(26):2757–64
162. Takaluoma K, Hyry M, Lantto J, Sormunen R, Bank RA, et al. 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(9):6588–96
163. Hyland J, Ala-Kokko L, Royce P, Steinmann B, Kivirikko KI, Myllylä R. 1992. A homozygous stop codon in the lysyl hydroxylase gene in two siblings with Ehlers-Danlos syndrome type VI. *Nat. Genet.* 2(3):228–31
164. van der Slot AJ, Zuurmond A-M, Bardoel AFJ, Wijmenga C, Pruijs HEH, et al. 2003. Identification of *PLOD2* as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. *J. Biol. Chem.* 278(42):40967–72
165. Salo AM, Cox H, Farndon P, Moss C, Grindulis H, et al. 2008. A connective tissue disorder caused by mutations of the lysyl hydroxylase 3 gene. *Am. J. Hum. Genet.* 83(4):495–503
166. Schegg B, Hülsmeier AJ, Rutschmann C, Maag C, Hennet T. 2009. Core glycosylation of collagen is initiated by two β(1-*O*)galactosyltransferases. *Mol. Cell. Biol.* 29(4):943–52
167. Ishikawa Y, Mizuno K, Bächinger HP. 2017. Ziploc-ing the structure 2.0: Endoplasmic reticulum-resident peptidyl prolyl isomerases show different activities toward hydroxyproline. *J. Biol. Chem.* 292(22):9273–82
168. Cabral WA, Perdivara I, Weis M, Terajima M, Blissett AR, et al. 2014. Abnormal type I collagen post-translational modification and crosslinking in a cyclophilin B KO mouse model of recessive osteogenesis imperfecta. *PLOS Genet.* 10(6):e1004465
169. Lietman CD, Rajagopal A, Homan EP, Munivez E, Jiang M-M, et al. 2014. Connective tissue alterations in *Fkbp10*$^{-/-}$ mice. *Hum. Mol. Genet.* 23(18):4822–31
170. Alanay Y, Avaygan H, Camacho N, Utine GE, Boduroglu K, et al. 2010. Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. *Am. J. Hum. Genet.* 86(4):551–59
171. Schwarze U, Cundy T, Pyott SM, Christiansen HE, Hegde MR, et al. 2013. Mutations in *FKBP10*, which result in Bruck syndrome and recessive forms of osteogenesis imperfecta, inhibit the hydroxylation of telopeptide lysines in bone collagen. *Hum. Mol. Genet.* 22(1):1–17
172. Lekszas C, Foresti O, Raote I, Liedtke D, König E-M, et al. 2020. Biallelic *TANGO1* mutations cause a novel syndromal disease due to hampered cellular collagen secretion. *eLife* 9:e51319
173. Wang Y, Liu L, Zhang H, Fan J, Zhang F, et al. 2016. Mea6 controls VLDL transport through the coordinated regulation of COPII assembly. *Cell Res.* 26(7):787–804
174. Pitman JL, Bonnet DJ, Curtiss LK, Gekakis N. 2011. Reduced cholesterol and triglycerides in mice with a mutation in Mia2, a liver protein that localizes to ER exit sites. *J. Lipid Res.* 52(10):1775–86
175. Baines AC, Adams EJ, Zhang B, Ginsburg D. 2013. Disruption of the *Sec24d* gene results in early embryonic lethality in the mouse. *PLOS ONE* 8(4):e61114
176. Garbes L, Kim K, Rieß A, Hoyer-Kuhn H, Beleggia F, et al. 2015. Mutations in *SEC24D*, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta. *Am. J. Hum. Genet.* 96(3):432–3