Elastic fibers are extracellular components of higher vertebrates and confer elasticity and resilience to numerous tissues and organs such as large blood vessels, lungs, and skin. Their formation and maturation take place in a complex multistage process called elastogenesis. It requires interactions between very different proteins but also other molecules and leads to the deposition and crosslinking of elastin’s precursor on a scaffold of fibrillin-rich microfibrils. Mature fibers are exceptionally resistant to most influences and, under healthy conditions, retain their biomechanical function over the life of the organism. However, due to their longevity, they accumulate damages during aging. These are caused by proteolytic degradation, formation of advanced glycation end products, calcification, oxidative damage, aspartic acid racemization, lipid accumulation, carbamylation, and mechanical fatigue. The resulting changes can lead to diminution or complete loss of elastic fiber function and ultimately affect morbidity and mortality. Particularly, the production of elastokines has been clearly shown to influence several life-threatening diseases. Moreover, the structure, distribution, and abundance of elastic fibers are directly or indirectly influenced by a variety of inherited pathological conditions, which mainly affect organs and tissues such as skin, lungs, or the cardiovascular system. A distinction can be made between microfibril-related inherited diseases that are the result of mutations in diverse microfibril genes and indirectly affect elastogenesis, and elastinopathies that are linked to changes in the elastin gene. This review gives an overview on the formation, structure, and function of elastic fibers and their fate over the human lifespan in health and disease.

Abbreviations
AA, allysine aldol; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; ADCL, autosomal dominant cutis laxa; AGEs, advanced glycation end products; ARCL, autosomal recessive cutis laxa; CCA, congenital contractural arachnodactyly; CL, cutis laxa; COPD, chronic obstructive pulmonary disease; DES, desmosine; EBP, elastin-binding protein; ECM, extracellular matrix; EDP, elastin-derived peptide; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERC, elastin receptor complex; FGF, fibroblast growth factor; GAGs, glycosaminoglycans; IDES, isodesmosine; IGF, insulin-like growth factor; LNL, lysinonorleucine; LOX, lysyl oxidase; LOXL, LOX-like; LTBP, latent TGFβ binding proteins; LTQ, lysine tyrosylquinone; Lya, allysine; MAGP, microfibril-associated glycoprotein; MASS, mitral valve prolapse, aortic enlargement, skin and skeletal findings; MD, molecular dynamics; MFS, Marfan syndrome; MMP, matrix metalloproteinase; Neu-1, neuraminidase-1; NMR, nuclear magnetic resonance; PCOS, polycystic ovary syndrome; PPCA, cathepsin A/protective protein; PXE, pseudoxanthoma elasticum; SANS, small angle neutron scattering; SAXS, small angle X-ray scattering; SVAS, supravalvular aortic stenosis; TE, tropoelastin; TGFβ, transforming growth factor beta; TFN, tumor necrosis factor; WBS, Williams-Beuren syndrome; WMS, Weill-Marchesani syndrome; XLCL, X-linked recessive CL; A-LNL, dehydrolysinoornitorleucine.
Introduction
A key step in the evolutionary process of vertebrates was the transition from an open to a closed, pulsatile circulatory system. This process involved adaptations of the blood vessel structure for an increased mechanical stability and elasticity to transport blood under high pressure [1]. The extracellular entities responsible for the elasticity and extensibility are the elastic fibers, which are highly abundant in large and small arteries. Elastic fibers store the potential energy required to maintain blood flow during diastole thus enabling proper cardiovascular function. Similarly, elastic fibers were significant in the development of energetically efficient lungs. The respiratory system in vertebrates stores potential energy in elastic tissues during inhalation, which is released as the lung thorax retracts during exhalation and used to drive lung recoil [2]. Elastic fibers are also crucial for the physiological function of numerous other force-bearing tissues and organs such as skin, ligaments, or bladder. The fibers are long-lasting macromolecular assemblies of the extracellular matrix (ECM) [3] of jawed vertebrates including cartilaginous fishes. They are composed of two morphologically distinct components: a dense core of crosslinked elastin, accounting for about 90% of the fiber content, surrounded by a mantle of longitudinally aligned microfibrils.

These microfibrils are 10–12 nm wide filaments that confer specific biomechanical properties to tissues, in part by the deposition of elastin onto a microfibrillar template. Fibrillins constitute the backbone of these microfibrils and are large (~ 350 kDa) modular organized glycoproteins with a remarkably high Cys content (~ 13%). From an evolutionary point of view, microfibrils are one of the oldest macromolecular ECM assemblies as they emerged more than 600 million years ago. They remained without significant changes until today [4] and are widely distributed from cnidarians to mammals [5,6]. Microfibrils provide elasticity to dynamic connective tissues over longer distances. Three fibrillin isoforms, encoded by separate genes, exist in humans (fibrillin-1 to fibrillin-3). All three fibrillins feature a multidomain structure with 47 epidermal growth factor-like (EGF) domains (all but five of which are calcium-binding), seven eight-Cys-containing domains and two further hybrid domains [7]. Despite their importance due to their structural properties, fibrillins interact with membrane receptors such as integrins and control growth factor signaling [8,9]. While the basic elements of microfibrils are fibrillins, a number of other proteins have been identified that interact with microfibrils during their biosynthesis [10]. Among them are microfibril-associated glycoproteins (MAGPs) [11,12], latent transforming growth factor-β binding proteins (LTBPs) [13–15], elastin microfibril interacers [16], a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and ADAMTS-like (ADAMTSL) proteins [17].

Unlike the family of fibrillins, which arose in early metazoans, elastin, the other key protein of elastic fibers, is neither present in lower chordates nor in any invertebrates [18]. Hence, it occurred evolutionary only recently (about 400 million years ago) and is a synapomorphy of gnathostomes. The cyclostomes, to which the extant lampreys and hagfish belong, are thought to have split off from other vertebrate lines before the evolution of bones and dentin and do not contain elastin. Interestingly, a protein referred to as lamprin has been found in lamprey. While it shares some characteristics with elastin, it also differs in many ways, including the crosslinks [19,20]. To date, the origin of elastin is unclear. It either appeared de novo or, more likely, has evolved from earlier proteins [21]. With regard to the latter hypothesis, it is worth noting a recent article that discussed the theory that elastin evolved from a preexisting fibrillin-1 [22]. Elastin imparts resilience and elasticity to most tissues subject to reversible deformation, as these properties are critical to their long-lasting functionality. It is a completely insoluble biopolymer made up of an indefinite number of units of its precursor tropoelastin (TE). The amino acid sequence of the soluble monomer is highly repetitive and consists predominantly (~ 78%) of the four amino acids: ~ 30% Gly, ~ 22.5% Ala, ~ 13% Val, and ~ 12.5% Pro (all values refer to human TE, isoform 2). With few exceptions, the amino acid residues of Trp, His, and Met are not found in TE [23].

In most vertebrate species, a single gene encodes TE. Exceptions known to date to possess two elastin genes are teleosts and frogs [24]. The human gene, ELN, is positioned on the long arm of chromosome 7 (q11.23) [25]. Its product, TE, is composed of hydrophobic and more hydrophilic domains arranged in a nearly alternating manner. Distinct exons encode for these domains, and hence, the domain structure reflects the gene’s exon organization (for detailed information on gene structure, see Ref. [21,26]). The domain structure is depicted in Fig. 1A. The hydrophobic domains account for the elastic and self-aggregation properties, whereas the hydrophilic ones participate in covalent crosslinking in the course of the maturation of elastin. The latter contain either Lys-Ala (KA) motifs, which are often surrounded by polyalanine stretches, or Lys-Pro (KP) motifs. The KA domains typically contain pairs or triplets of Lys...
residues with two or three Ala residues and occasionally another residue between them. In KP domains, there is at least one Pro residue and often another residue between two Lys residues. Compared to the elastin genes of other vertebrates, the human gene has the peculiarity of lacking exons 34 and 35, which have been lost in the course of the evolution of primates (nonhuman primates only lack exon 35) [27]. The ELN primary transcript is subject of substantial alternative splicing leading to the production of several TE isoforms and, thus, to an increase in diversity. Human TE, for example, is represented by at least 18 theoretical isoforms with molecular masses of 49–69 kDa [26,28]. The splicing mostly takes place in a cassette-like fashion, and thus, an exon is either deleted or included. However, it also is possible for an exon to be divided by a splicing event [29]. There is inconclusive information about the exact number of exons involved, but clearly the exons encoding the hydrophobic domains 3, 11, 22, 32, and the hydrophilic domains 10, 13, 23 are alternatively spliced (possibly more) [26,30–33]. A particular case is exon 22, which always seems to be spliced out in humans [23]. The exclusion of the respective hydrophobic domain results in the union of the crosslinking domains 21 and 23 (see Fig. 1A). Several studies have proven the expression of numerous isoforms of human elastin on the mRNA or protein level [30,34]. The specific impacts of alternative splicing on the structure and functioning of elastin are still elusive. There are studies suggesting a connection with the tissue type or a correlation with development-related changes, which indicates the importance of alternative splicing for elastogenesis [35–38]. Moreover, it has recently been shown at the molecular level that changes of TE’s domain structure or the exchange of specific residues alter the coacervation and crosslinking processes. This, in turn, affects TE’s assembly into a biopolymer as well as its resultant biomechanical characteristics. It supports the theory that TE variations enable tissue-specific changes of the properties of elastin or are the cause of impaired elastogenesis under disease conditions [39–41].

Tropoelastin is secreted into the extracellular space as nonglycosylated protein by elastogenic cells such as fibroblasts [42], smooth muscle cells (SMCs) [43], endothelial cells [44,45], chondrocytes [46], and keratinocytes [47]. Depending on the organ and tissue and its mechanical requirements, the mature fiber networks differ highly in their structural configurations, for example, concentric fenestrated lamellae in aortic tunica media, reticular fiber networks in the dermis, or spatial honeycomb-like structures in elastic cartilage. Table 1 gives an overview of various morphologies of elastic fiber networks in some human tissues and the respective elastin contents. It is evident that the configurations are linked to the extent and direction of mechanical forces exerted on the connective tissues. Also related to this is the elastin content, which varies greatly (2–75%) between the tissues, but also between identical tissues of different species [48–55]. In spite of its low amount in some tissues such as skin or intervertebral disk [52,56], elastin is essential for conferring tensile strength and elasticity.

Elastogenesis

The formation of elastic fibers follows a complex multistage process involving interactions of the key proteins with numerous other proteins and molecules and is referred to as elastogenesis. The major steps of the currently accepted model for elastogenesis are depicted in Fig. 1B. The two morphologically distinguishable components of which the elastic fiber is composed, the elastin core and the microfibrillar sheath, are formed independently of one another and are also discussed separately below. The nature as well as the structural and functional consequences of most of the underlying interactions are so far only partially understood. It is therefore possible that the further described elastogenesis proceeds, at least in part, differently with regard to the location and order of the respective processes. For example, there is new evidence that some initial steps of assembly and crosslinking already take place within the cell [57].

Formation of microfibrils

The elastogenesis starts with the formation of microfibrils that are assumed to serve as a scaffold for the successive TE deposition. The microfibrils are mainly composed of fibrillins [58], whose monomers are secreted by mesenchymal cells such as SMCs and fibroblasts. Regardless of the tissue, fibrillin-1 is the primary component of microfibrils. While fibrillin-1 is produced throughout life, fibrillin-2 and fibrillin-3 are mainly expressed in fetal tissues [59–61]. However, the expression of fibrillin-2 is upregulated in wound healing and sclerosis [62]. While the precursors, the profibrillins, are being secreted or thereafter, they undergo proteolytic processing at their N and C termini by furin [63,64]. These cleavages are part of the maturation leading to the ~320 kDa large forms and are further essential for the ensuing linear multimerization through the proteins’ C termini into beaded structures and their assembly at the cell membrane [65,66]. Moreover, the N-terminal propeptide precludes premature
intracellular assembly of fibrillin-1 and is thus important for its secretion [67]. It has been experimentally shown that the assembly to multimers enhances the interaction of the beads with the N terminus of fibrillin-1, with fibronectin, and with heparin/heparan sulfate [66,68]. It is suggested that the fibrillin beads first bind to those proteoglycans at the cell membrane that contain heparan sulfate and subsequently undergo
a fibrillin-1-mediated focal adhesion formation. In the following, the N and C termini of the fibrillin monomers interact with each other, leading to the alignment of the fibrillin monomers in a parallel head-to-tail manner and their lateral interaction [69,70]. The growing microfibrils with a ‘beads-on-a-string’ appearance are then transported to a fibronectin network, which is assumed to be important for their stabilization and/or fostering their interplay with other microfibril proteins [68]. The three-dimensional bundle structure of fibrillin networks can be further stabilized by intermolecular disulfide bonds [71] and transglutaminase-mediated ε-(γ-glutamyl)lysine crosslinks [72,73]. Fibrillin-1 is also capable of cellular interactions via integrins such as α5β1, α6β3, and αvβ6 [74,75]. However, it is not yet known what significance this interplay has for elastogenesis [74].

Synthesis and secretion of tropoelastin

As stated above, TE’s pre-mRNA is subject of significant alternative splicing, resulting in the generation of multiple isoforms. Translation of the mRNA takes place on the surface of the rough endoplasmic reticulum where also cleavage of the signal peptide occurs. Prior to transport of the elastin precursor through the Golgi apparatus to the cell membrane, ~10% of the Pro residues are hydroxylated by prolyl 4-hydroxylase. In contrast to collagens, where the function of hydroxyproline, that is, the stabilization of the triple-helix, is well defined, the impact of this post-translational modification on the structure and function of elastin is yet unknown. However, there is evidence that a high degree of hydroxylation affects the thickness and elasticity of elastic fibers [78] as well as their susceptibility toward enzymatic degradation [76]. Other results suggest that elastin’s hydroxylation degree may represent a possibility to molecularly adjust its properties to the tissue-dependent functional and mechanical requirements [79]. In any case, the presence of this modification, as well as the different isoforms and the crosslinking discussed below, significantly increase molecular diversity.

Intracellularly, TE binds to a chaperone, the elastin-binding protein (EBP), which is a β-galactosidase splice variant with a molecular mass of ~67 kDa [80]. Its function in elastogenesis is to protect TE from unwanted intracellular degradation and coacervation. It also mediates its delivery to the cell membrane [81,82]. After secretion of the complex of TE and EBP, the latter interacts with two other proteins

Table 1. Morphological configurations of elastic fibers in several tissues and their elastin contents.

| Human tissue                   | Elastin content | Morphological configuration | Reference |
|--------------------------------|-----------------|-----------------------------|-----------|
| Aorta (tunica media)           | < 57%           | Concentric fenestrated lamellae | [48,49]   |
| Elastic cartilage              | 19%             | Honeycomb-like structures    | [50]      |
| Elastic ligaments              | < 75%           | Rope-like fibers             | [51]      |
| Intervertebral disk (anulus fibrosus) | 2%             | Densely clustered and randomly oriented | [52] |
| Intervertebral disk (nucleus pulposus) | 2%             | Radial fiber network         | [52]      |
| Lung (parenchyma)              | < 30%           | Lamellar sheets surrounding the alveoli | [53] |
| Lung (airways mucosa)          | < 5%            | Crimped lamellar organization | [281,282] |
| Skin (dermis)                  | < 5%            | Rope-like fibers             | [54]      |
| Tendons                        | 4%              | Bundles of long fibers       | [51,55]   |

*aPercentage of the dry weight.; bContent determined for bovine elastin.

![Fig. 1.](http://example.com/fig1.png) (A) Scheme of the exon and domain structure of human TE. The numbering is based on exon assignment and indicates crosslinking domains (numbers on top; light and dark red squares) and hydrophobic domains (numbers below; gray squares). Domains encoded by exons subject to alternative splicing are indicated by filled circles. The sequence motifs of the eleven Lys-Ala and five Lys-Pro crosslinking domains are shown above the scheme. The width of the squares corresponds to the relative sequence length of the respective domains. (B) The stages of elastogenesis: (1) Fibrillins and other proteins associated with microfibrils are secreted, multimerize in the ECM, and are assembled to a microfibrillar template. (2) The TE monomers are synthesized on the rough endoplasmic reticulum (ER) where they associate with their chaperone, the EBP. (3) This complex is transported through the Golgi apparatus and secreted to the plasma membrane. (4) The subsequent dissociation of the EBP-TE complex is induced by the binding of GAGs to EBP. The released TE molecules aggregate at the cell surface. Fibulin-4 mediates the proper alignment of TE molecules and their interaction with lysyl oxidases. This step leads to oxidative deamination of Lys residues and induces a sequence of condensation reactions giving rise to intra- and intermolecular crosslinks. (5) The premature elastin clusters grow through the addition of TE molecules and detach from the cell surface as they reach a critical size. Fibulin-5 guides the premature elastin to the microfibrillar scaffold. (6) The elastin clusters coalesce into larger aggregates and are crosslinked further, completing the maturation of the elastic fiber.
bound to the cell membrane: the transmembrane sialidase neuraminidase-1 (Neu-1) and the protective protein/cathepsin A (PPCA). EBP, PPCA, and Neu-1 are the subunits that form together a heterotrimer, the elastin receptor complex (ERC) [83]. The subsequent binding of glycosaminoglycans (GAGs) to the galactolectin site of EBP is thought to cause changes in the conformation of EBP, which in turn triggers the release of TE from the complex [81,82]. The chaperone is not degraded, but recycled back into the intracellular endosomal vesicles, where it binds to another elastin monomer, and thus, the process is repeated. The reuse of EBP is considered an essential part of elastogenesis [84].

**Microassembly**

The release of TE is followed by a reversible self-assembly, termed coacervation [85]. In this entropically driven, endothermic process, hydrophobic regions such as domains 17–27 of TE interact with each other [86]. The coacervation results in the formation of distinct spherical aggregates attached to the cell surface [87,88]. The process can be reproduced and studied *in vitro* using solutions of recombinant TE or TE-derived polypeptides, which become turbid when a critical transition temperature is reached [89,90]. The aggregation rate, the size, and other properties of the globules are influenced by the peptide or protein concentration, temperature, pH, and ionic strength [91]. In recent work, Reichheld *et al.* [92] proposed a model based on data from solid-state and solution NMR studies that describes the structure and dynamics of an elastin-like polypeptide during self-assembly including liquid–liquid phase separation and subsequent crosslinking. According to this model, hydrophobic collapse and, thus, phase separation occurs due to an elevated entropic penalty of solvating the polypeptide chains under increased salt concentration and temperature. The inability of the hydrophobic domains rich in Pro and Gly to form stable secondary structures [93] maintains disorder in the coacervated state. Crosslinking occurs after exclusion of bulk water and reduction of Na⁺ and Cl⁻, which further enhances coacervation [92]. Other studies suggest that TE’s C-terminal region, particularly domain 30, may interact with microfibril-associated proteins, and thus, it could be crucial for elastogenesis [94,95]. The C-terminal domain 36, which contains the polybasic sequence motif KXXXRRKK that is highly conserved among species, also appears to be important for elastic fiber assembly. Although its exact role has not yet been elucidated, it has been shown *in vivo* that TE lacking this part of the sequence is less efficiently transformed into mature elastin and exhibits abnormal crosslinking [94,96]. It is possible that proteoglycans associate with this domain and in this way mediate the necessary alignment of the TE monomers [97]. Moreover, GAGs have been shown to accelerate the coacervation process and spherule fusion through dominant charge interactions involving side chains of Lys residues [98,99]. Another important role is played by fibulin-4, which mediates the alignment of TE molecules and their following interaction with the copper-dependent lysyl oxidase (LOX) [100–103].

**Crosslinking and macromolecular assembly**

Similar to collagens, the formation of crosslinks in elastin is initiated by the action of members of the lysyl oxidase family. In addition to LOX, four lysyl oxidase like enzymes (LOXL1-4) belong to this family. According to their homology, they can be categorized into two subgroups: (a) LOX and LOXL1 and (b) LOXL2, LOXL3, and LOXL4. Members of the second subgroup possess a propeptide region with four conserved scavenger receptor cysteine-rich domains, which are likely to mediate interactions between matrix proteins [104,105]. All five enzymes feature the amine oxidase domain at the C-terminal region including copper-binding residues and the lysine tyrosylquinone (LTQ) cofactor required for the activity of this catalytic domain (reviewed in [106]). Recently, the matricellular protein fibulin-4 was shown to be essential for the formation of LTQ and thus for the activation of LOX [107].

LOX and LOXL1 have been shown to be vital for inducing the crosslink formation between TE molecules [108,109]. Moreover, LOXL2 was shown to interact with TE *in vitro*, resulting in the typical elastin crosslinks. *In vivo*, both proteins were found to be co-distributed in the vessel wall, suggesting that LOXL2 may contribute to elastin maturation [110]. After secretion of LOX and LOXL1 as inactive forms, they are subject to proteolytic cleavage by bone morphogenetic protein-1/procollagen C-proteinase (BMP-1) [111]. The active enzymes subsequently catalyze the oxidative deamination of the ε-amino group of peptidyl Lys residues to the highly reactive ε-aminoacidic acid-δ-semialdehyde, also known as allysine residue (further denoted as Lya). The Lys residues are found in the hydrophilic crosslinking domains. For humans, these are 11 KA domains and five KP domains (Fig. 1A). After oxidation, crosslinks are spontaneously formed within and between TE molecules by nonenzymatic condensation. Two Lya residues can react with each other via aldol condensation, resulting in the
formation of allysine aldol (AA). A second type of bifunctional crosslink, dehydrolysinonorleucine (Δ-LNL), is formed when a Lya residue condenses with the ε-amino group of an unmodified Lys residue via Schiff base reaction [112,113]. Those reducible crosslinks (AA and Δ-LNL) subsequently condense with each other, partly with involvement of unoxidized Lys residues or with other intermediates, to form the trifunctional crosslinks dehydromerodesmosine (reducible to merodesmosine) and cyclopentenosine [114–116], as well as the tetrafunctional isomeric crosslinks desmosine (DES) and isodesmosine (IDES) [117,118]. In addition, some studies have shown the formation of pentafunctional crosslinks, which include pentasine and allodesmosine. [118,119]. The formation pathways and structures of the major crosslinks are depicted in Fig. 2. Interestingly LNL and AA are also found in collagens, whereas DES and IDES are unique to elastin.

Fig. 2. Formation of the most important elastin crosslinks. The enzyme lysyl oxidase accounts for the initial step. It oxidatively deaminates side chains of Lys residues, producing the reactive aldehyde allysine by consuming oxygen and releasing hydrogen peroxide and ammonia. The subsequent reactions are spontaneous and proceed without the involvement of the enzyme. The aldehyde groups of two allysine residues condense to AA. Alternatively, one allysine residue reacts with another Lys residue to form dehydrolysinonorleucine via Schiff base reaction. Further condensation of these bifunctional crosslinks or their reaction with other intermediates, including the trifunctional crosslink merodesmosine, yields the unique tetrafunctional crosslinking amino acid isomers desmosine and isodesmosine.
elastin in mammals [120–123]. However, DES and IDES are also present in avian and reptilian eggshell membranes [124,125].

On the cell surface, the cluster of premature elastin continues to grow through the addition of TE molecules and their subsequent crosslinking until it eventually detaches and moves through the extracellular space. Interactions of fibulin-4 and fibulin-5 with fibrillin are likely to mediate the regulation of elastin globules onto the microfibril network [103,126,127]. The binding of fibulin-5 to fibrillin-1 leads to a potentiation of the binding between this glycoprotein and TE by acting as an adaptor [128]. Further involved is LTBP4, which interacts with the complex of the two fibrulins and TE and appears to be essential for the proper linear deposition of TE on the microfibril scaffold [129–131]. As this association between fibrillin-based microfibrils and the elastin aggregates proceeds, the latter coalesce into larger assemblies, eventually giving rise to functional fibers. The crosslinking, that is, oxidation by lysyl oxidases and subsequent condensation reactions, continues afterward and further stabilizes the newly formed fibers. This presumably last step of elastogenesis proceeds remarkably slowly [132].

**Postnatal decline in elastin synthesis**

The production of functional elastic fibers is governed by dynamic regulation and occurs over a defined and relatively short duration. In most mammals, the expression of TE starts late in the fetus life, reaches very high levels during the neonatal stages, and decreases after birth before it eventually ceases completely [133,134]. The turnover of elastin is extremely slow, and there is virtually no new elastogenesis in adult life [135,136]. A remarkable exemption, however, is the uterus: Elastogenesis starts anew with each pregnancy and is followed by the quick removal of elastin just after parturition [137].

Regulatory control varies by cell type through tissue development. For instance, the production of TE in the lungs first undergoes postnatal downregulation, then upregulation during the alveolarization phase and is later attenuated again [138]. Another example is the temporary reactivation of the expression as a result of injuries or pathologic conditions such as solar elastosis or severe chronic obstructive pulmonary disease (COPD). However, the assembled fibers usually have only limited functionality [139,140]. Studies show that transcriptional and post-transcriptional machineries control elastin production [32,141]. The cytokines known for influencing the regulation can be categorized into proelastogenic factors including transforming growth factor β-1 (TGFβ1), insulin-like growth factor-I (IGF-I), and anti-elastogenic factors. The latter include basic fibroblast growth factor (bFGF/FGF-2), heparin-binding EGF-like growth factor (HB-EGF), EGF, platelet-derived growth factor-BB (PDGF-BB), TGFα, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and noncanonical TGFβ1 signaling (for an overview, see [142]). While several positive as well as negative effectors have been determined in experimental studies, the specific involvement of these cytokines, additional messengers, or autoregulatory processes in elastogenesis remains elusive. It is reasonable to assume that several factors interact contemporaneously with elastogenic cells *in vivo* and participate in the precise regulation of the dynamic and complex course of elastic fiber formation.

**Properties of elastin**

**Durability and proteolytic resistance**

Native elastin is exceptionally resistant to a wide range of influences, which is linked to its composition, the extremely dense packing of the molecules and the high degree of covalent crosslinking. Provided elastic tissues are not affected by diseases, elastin is metabolically stable. Analyses of human elastin by Asp racemization and measurements of 14C levels in postmortem tissues revealed a remarkable half-life of over 70 years [143,144]. This high durability is related, among other things, to elastin’s high resistance to proteolytic degradation. Unlike most other proteins, mature elastin is not cleavable by trypsin due to the absence of the respective residues (Arg) or their involvement in cross-links (Lys). Nevertheless, some of the many extracellularly acting proteinases are capable of digesting crosslinked elastin. As discussed in more detail below, the structural integrity of elastin can be impaired by these enzymes, especially when they are upregulated in the course of diseases, but also by other causes such as excessive mechanical stress. When studying elastin, the elastolytic potential of proteinases, or the liberation of EDPs *in vitro*, the knowledge about the substrate’s initial condition is of utmost importance. However, this information has often been neglected in past studies. The isolation procedure used for the purification can damage elastin and cause cleavage and disintegration of the elastin core [145,146]. Therefore, the purification method should be selected carefully to suit the respective study and substrate requirements. In addition to various classically used and rather harsh methods including the hot alkali technique [147] or the Starcher...
method [148], several gentle methods have been published more recently [146,149]. They allow obtaining virtually intact fibers that still possess their fibrillar conformation. However, these so-called ‘elastin fibers’ are devoid of the microfibrillar sheath, which is lost in the course of all known isolation procedures.

Figure 3A illustrates age-related effects on elastin integrity by means of isolated fibers from the skin of two subjects of very different ages (6 vs. 90 years). Although the fibers have similar geometries and are ~ 2 µm in diameter, they exhibit otherwise very different morphologies. While the fibers of the young subject appear completely intact and have smooth surfaces, those of the old subject are severely damaged, show cracks, and partially disintegrate. More important than appearance, however, is that this damage is also associated with reduced stability of the fiber protein to proteases acting extracellularly; a weakness that finds resonance during aging [146]. Indeed, as an organism ages, a chronic low-grade inflammation occurs (inflammaging). This, associated with the inexorable mechanical fatigue of elastin, weakens the elastin network and makes it more prone to degradation in a vicious circle manner. This could explain the increased decay of elastic fibers and their progressive loss of function in old age.

### Structural properties

Macroscopically, elastin appears as an amorphous, pale-yellowish material. Investigations on the ultrastructural level, using different microscopic techniques, reveal its fibrillar organization and the presence of fine filaments with diameters of ~ 5 nm that are parallel-aligned and form a spatial network along the fiber [150–152]. Data of these and other studies further indicate that the filaments are laterally associated and organized in an ordered array. Due to its high hydrophobicity and related insolubility, structural studies of elastin at the protein level represent a major challenge. One way to make elastin at least partially accessible for analytical studies requires its prior partial hydrolysis through the use of elastin-degrading proteases or chemical reagents. Chemical breakdown can be achieved, for example, by applying oxalic acid or potassium hydroxide resulting in the soluble α and κ-elastins, respectively [153]. In the past, various studies have been conducted aiming to elucidate the secondary structure or secondary structural elements in various elastins, elastin peptides, and TE. Studies based on circular dichroism (CD) measurements of full-length TE revealed secondary elements of 3% α-helix, 21% β-turn, 41% β-sheet, and 33% undefined

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**Fig. 3.** (A) Scanning electron microscopic images of purified dermal elastin fibers from young and aged subjects (6 and 90 years, respectively). While the fibers of the young subject appear smooth and intact, those of the aged subject are highly fissured and exhibit ruptures. (B) Enzymatic and nonenzymatic aging processes in elastin and their consequences. During aging, several enzymatic and nonenzymatic processes affect elastin. The observed loss of mechanical function is associated with elastokine production fundamentally affecting cardiovascular diseases but also cancer-related processes.
structure [90]. In contrast, a study on insoluble elastin published shortly thereafter and conducted using various spectroscopic methods as well (Fourier-transform infrared spectroscopy, near-infrared Fourier-transform Raman spectroscopy, and CD) showed a secondary structure composition of 10% α-helix, 35% β-strand, and 55% undefined conformations [154]. The origin of the always found α-helical conformations lies in the polyalanine sequences of the KA domains. The helices are presumably formed upon coacervation and most likely necessary for the formation of bifunctional intramolecular crosslinks, which subsequently give rise to higher functional crosslinks such as DES or IDES through further condensation reactions. Hence, it can be inferred that α-helices must remain throughout elastogenesis and are also present in mature elastin [155]. The flexibility of TE’s backbone, as well as the large size of the molecule, limits so far the use of NMR spectroscopic methods for resolving the structure [156]. However, one can assume that this will become possible in the future with further instrumental improvements. Furthermore, there has been no success in attempting to crystallize full-length TE. Possible reasons for TE’s inability to form a uniform crystal lattice are its flexible structure, the self-assembly behavior, and the existence of concurrent conformations in TE. However, as mentioned before, low-resolution three-dimensional data of hydrated but unmodified human TE and some derivatives were obtained from small angle X-ray (SAXS) and neutron scattering (SANS) experiments [157]. The findings indicate an asymmetrical shape for unmodified TE, which appears to be composed of at least three functional elements. These include a coil region, important for elastic properties, a cell binding C-terminal region, and, the third component, a highly exposed bridge region that links the two others and is thought to be essential for elastogenesis [158].

To date, it has not been possible to elucidate the complete crosslinking pattern of elastin. As mentioned above, α-helical conformations dominate KA domains. This has the effect of locating the side chains of the Lys residue pairs on the same side of the helix, thereby enhancing the crosslinking process [155]. As it can be seen in Fig. 2, the formation of DES/IDES requires that one of the four Lys residues involved retains its ε-amino group [159]. This is probably aided by bulky hydrophobic residues such as Tyr, Phe, Leu, or Ile, whose presence adjacent to the respective Lys residues (see Fig. 1A) might impede or prevent its oxidation by amine oxidases [160,161]. The Pro residues in KP domains hinder the formation of α-helices [162], and KP domains are, moreover, often flanked by bulky hydrophobic amino acids such as Val, Ile, and Leu. The corresponding Lys residues are thus potentially less susceptible to oxidation and have been assumed to form crosslinks other than DES/IDES [163]. However, this hypothesis is inconsistent with the observation that the DES and IDES contents of species such as salamander or frog, which possess many KP domains, are about the same as in teleost elastins [18], in which KA domains predominate [23]. Moreover, Baig et al. [164] identified a tetrafunctionally crosslinked peptide from bovine nuchal ligament, where one of the peptide strands originated from a KP domain.

Brown-Augusburger et al. [163] elucidated the first crosslinking sites in elastin isolated from the aortas of copper-deficient pigs. The deficit of copper led to a decreased LOX activity in the developing animals and consequently to impaired crosslinking of elastin. This resulted in a reduced resistance to proteolysis, to which the protein was subjected for further investigations after purification. By investigating the digests, the authors found a peptide containing residues from three TE chains linked with each other by three crosslinks. One DES had formed between the domains 19 and 25 of two antiparallel chains. These KA domains are particular in that they are the only ones in human elastin in which three Lys residues are present (Fig. 1A). The two other crosslinks were LNLs and had formed between the two remaining Lys residues of these two KA domains and one Lys residue each of KP domain 10 of the third TE chain [163].

Based on these porcine elastin crosslinks as well as the low-resolution structure of recombinantly produced human TE obtained from SAXS and SANS investigations, Baldock et al. suggested that the polymerization of TE occurs in a head-to-tail fashion [157]. In this model, monomer units align themselves by convergence of the head and tail regions with subsequent assembly and polymerization in a tandem orientation to promote elasticity. It is worth mentioning that the formation of this string of TE molecules would require only one KP domain (10) and two KA domains (19 and 25) of the total 16 crosslink domains in the precursor. Hence, such an assembly could only represent an intermediate in the course of elastogenesis. Presumably, these primary fibrils must laterally associate with each other via hydrophobic interactions. Further crosslinking involving other KA and KP domains would ultimately result in the formation of larger assemblies [157]. It remains to be proven whether this model adequately describes the assembly of the TE molecules in vivo.

Attempts to shed light on the TE assembly on the molecular level were recently made by in-depth
characterizations of the crosslinking pattern of bovine [165] and human elastin [28] using state-of-the-art mass spectrometry [166], in-house developed software, as well as amino acid analyses. The results show that the crosslinking pattern of mature elastin is much more complex and random than previously assumed, with mature elastin being composed of an unordered network of TE molecules. In fact, when analyzing specific Lys residues in different TE chains, they were found to be both completely unmodified and involved in various types of inter- and intramolecular cross-links, which all coexist in mature bovine and human elastin [28,165]. In both elastins, the abundance of free Lys was very low (11% in human; 8% in bovine), proving that the crosslinking degree was generally high. KP domains were nearly exclusively crosslinked via LNL, which supports earlier hypotheses that they are not involved in DES/IDES formation [163], whereas KA domains were found to be crosslinked via LNL, AA, and DES/IDES. It has been hypothesized that AA may only form when two Lys residues are separated by three Ala residues (KAAK motifs), whereas the smaller Δ-LNL and its reduction product LNL form when two Lys residues are separated by two Ala residues (KAAK motifs) [161]. Data on human elastin revise this hypothesis as LNL crosslinks were also identified in domains with KAAK motifs [28]. With regard to the formation of tetrafunctional crosslinks, it was confirmed that KA domains 15, 27, and 17 or 31 in bovine elastin [165] and KA domains 6, 14, 17, 19, 25, 27, and 31 in human elastin [28] are involved in formation of DES/IDES, respectively. A DES/IDES peptide involving domain 14, which contains only one Lys residue and requires at least two more domains to form DES/IDES, demonstrates that tetrafunctional crosslinks can be formed in vivo through the involvement of three domains [28]. This finding revises previous assumptions that the formation of DES or IDES requires the condensation of an AA, involving two Lys residues on one chain, with an intramolecular Δ-LNL formed between a Lya and a Lys residue on a second chain [167–169]. A formation deviating from this is also consistent with in vitro experiments that demonstrated the formation of DES/IDES from three short elastin peptides containing KA domains [170].

Overall, recent findings show a randomized crosslinking pattern of different elastins [171]. It is evident that involved lysyl oxidases can basically access all Lys residues of KA and KP crosslinking motifs and transform them to Lya in a stochastic manner, even those followed by a bulky residue. This was shown by the elucidation of peptides crosslinked by AA in domains with bulky residues [28], since the formation of AA requires the oxidation of both Lys residues. However, most peptides originating from KA domains with KF, YK, or KY motifs were crosslinked via LNL, which indeed indicates reduced activity of LOX for Lys residues next to the bulky, aromatic residues Phe and Tyr as postulated previously [160,161].

Secondary structure and mechanical properties

The aorta in humans absorbs the impact of about 3 billion heart beats during an average lifespan. The vessel walls withstand the load without rupture due to the presence of elastic fibers, which distribute the repetitive mechanical stress in the tissue. Elastic fibers provide vertebrate tissues with remarkable mechanical properties and are more flexible and elastic than other ECM assemblies. Compared to collagens, for example, which in the hydrated state are relatively inelastic with Young’s moduli of between 1 and 2 GPa [172], the elastic moduli of single elastic fibers lie between 300 and 1500 kPa [173]. Thus, elastic fibers can be linearly extended more than twice their length (~150%) before rupture occurs [174] and, once tension is released, they return to their original dimensions without any signs of hysteresis. Collagen fibers, on the other hand, can be stretched only insignificantly (~13%), but they have a high tensile strength of 0.12 GPa, which is with ~2 MPa about 60 times lower for elastin [174–176]. The resilience of both proteins is very similar (~90%) [174]. It is important to note that elastin needs to be hydrated in order to possess its elastomeric properties [177]; dried elastin is of hard and rigid nature.

A few models explain the biopolymer’s mechanical properties based on the change of entropy of the system. The first one of these models, introduced by Hoeve and Flory in the fifties [178], states that the increase in entropy drives elastin contraction after stretching [174,178]. Covalent crosslinks distribute the stress and strain throughout elastin during the stretch and induce organization within its core. The stress release, on the other hand, increases entropy to its maximum. The same authors later proposed a simple isotropic model based on elastic fibers as a network of random chains [179], which, however, fails to explain why elastin requires water for elasticity. The main difference among the several structural models proposed to describe the maintenance of elastin flexibility is the nature of the ordered structures that contribute to the entropic properties of the molecule.

Large secondary structures are not likely to be formed in TE, as it contains a high number of the disorder-inducing residues Pro (12%) and Gly (30%)
However, some more recent anisotropic models elucidate the contribution of short and labile β-turns (types I, II, and VIII) and polyproline II helices [180] in elastin to explain its properties. The ‘librational entropy model’ introduced by Urry et al. [181] proposes that elastin molecules fold into flexible β-spiral regions consisting of consecutive β-turns of type II. However, this model lacks experimental evidence and is not supported by molecular dynamics (MD) simulations [182,183]. Based on experimental results, more recent work proposes that the entropy increase occurs due to the formation of labile β-turns and their sliding along the chain [184,185]. However, none of these and further models completely explain both the elasticity and the necessity for elastin’s hydration for its function. Interestingly, MD simulations of elastin-derived peptides (EDPs) and solid-state NMR experiments with mature elastin brought new insights about the high degree of dynamic disorder in hydrated elastin in the relaxed state [182,186]. Experimental results presented in several works point to elastin flexibility being dependent on distinct dynamics and not on a rigid structure. A complete understanding of the precise mechanism behind elastin’s flexibility is still lacking. It requires further structural studies to elucidate the complex crosslinking pattern of elastin and the inclusion of such data in future MD simulations.

**Biological significance, aging, and diseases**

**Biological functions**

Elastin is a vital component of vertebrates. Its foremost biological role is to confer elasticity and resilience to tissues so that they can persistently withstand mechanical forces and deformations and perform their designated tasks. Without elastic fibers, the mechanical function of the lungs or the pulsatile circulatory system would not be possible. In addition to the prominent structural roles, by which it co-determines the architecture and biomechanical properties of the ECM, elastin has also regulatory functions during arterial development [187] and plays roles in various physiological processes [188]. While elastin is an exceptionally resistant component of the ECM, it is subject to changes and damages during the life of an organism, in particular during aging or pathological processes described in the following sections. Inflammatory conditions such as atherosclerosis [189], COPD [190,191], and aneurysm formation [192] are known to be linked to elastin depletion and its serious consequences. A hallmark of elastin degradation is the release of EDPs, some of which are able to enter the bloodstream. Those EDPs that possess biological activities are also referred to as elastokines. They belong to the group of matrikines, which are bioactive peptides released from ECM proteins.

So far, three receptors of EDPs have been described [193], of which the best known is the already mentioned ERC, a protein complex consisting of PPCA, Neu-1, and EBP. Besides EBP’s chaperone function for TE during elastogenesis, it is also known to interact with elastokines, which stimulates the sialidase activity of Neu-1. This in turn is most likely crucial for the intracellular signal transduction causing the initiation of several signaling events that vary greatly between the various cell types [194]. The ERC foremost recognizes elastokines with the consensus sequence XGXXPG that is known to promote the formation of β-turns of type VIII, which is required for binding to EBP [82,195]. The second elastokine receptor is galectin-3 (~ 30 kDa), a laminin/elastin-binding protein and member of the lectin family with broad biological functions. Galectin-3 is associated with numerous pathologies and important for interactions between the extracellular space and cells [196]. Furthermore, several integrins of the αV-family (αVβ1, αVβ3, and αVβ5) have been also described to interact directly with TE and/or with elastin peptides [197–200]. The cell attachment to TE, for example, via its C-terminal RKRK motif, is crucial to elastogenesis as the cells play important roles in determining the organization of the fibers into three-dimensional structures. Further, the interaction of integrins with degradation products of elastin may account for some of the effects triggered by elastokines. They have been shown to alter the cellular physiology of different cell lines including fibroblasts and leukocytes [201], and several studies have reported that elastokines possess a broad range of biological functionalities. That will be exemplified later in this work with a focus on their involvement in cardiovascular diseases and cancer.

**Aging**

During aging, elastin undergoes diverse types of detrimental alterations ranging from mechanical fatigue, chemical modifications to enzymatic degradation that deeply modify the macroscopic aspect of the fibers (Fig. 3). Elastin fatigue is a concept that has been evidently described many years ago in the context of vascular biology, although it can be extended to other tissues. Elastin undergoes billions of oscillations during life leading to its inexorable degradation and to a loss of elasticity that causes a stiffening of the tissue.

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**References**

[93].
Elastin fatigue has been described to originate from repeated stretching of elastic fibers or from repeated cycles of shear stress within and between the elastic fibers. In both cases, the consequence of this mechanical degradation is the transfer of the mechanical load bearing from elastin to collagen [202,203].

As one of the longest-lived proteins in vertebrates, elastin can suffer diverse nonenzymatic modifications, which can potentially impair its functionality and that of the respective tissue. To these chemical modifications belong glycation and carbamylation [204,205], which are post-translational modifications exacerbated during pathologies such as diabetes or chronic kidney diseases, and are thought to modulate elastin stiffness besides their potential role in chronic inflammation.

Glycation is a process resulting in the formation of advanced glycation end products (AGEs). One of the underlying causes is the complex Maillard reaction, by which reducing sugars react with amino groups in proteins. This chemical reaction has been demonstrated almost 50 years ago to occur on hemoglobin A1c (HbA1c) during diabetes and for which it is one of the main causes of associated complications [206]. Three types of reactions have been described depending on the kinetics of the reaction [207]: (a) the formation of Schiff bases and their conversion to Amadori product (fructosamine); (b) the formation of reactive free α-dicarboxyl glyoxal compounds [208] that cause the crosslinking of target proteins; (c) the late Maillard reactions that lead to formation of crosslinks from glycated proteins, the AGEs [209]. The latter has been demonstrated in vivo and shown to be generated through multiple pathways (oxidative and nonoxidative reactions, that is, glycation and glycoxidation, respectively) [210]. Bearing in mind the extremely long half-life of elastin, its possibility to be subject of glycation throughout life is considered very high. Interestingly, it has been clearly shown that elastin from young individuals is more prone to form both fructosamine and AGEs under glycation in vitro than elastin from older individuals. Even if the functional consequences of these modifications are yet unclear, it can be hypothesized that the formation of such molecular adducts plays a role in ECM chronic inflammation observed during aging and metabolic diseases [211].

Recently, elastin has been demonstrated to be susceptible to another nonenzymatic modification, carbamylation. This reaction corresponds to the nonenzymatic binding of isocyanic acid. This mainly originates from urea decomposition, a process which is intensified during chronic kidney disease, but also in the course of vascular pathologies such as atherosclerosis involving the enzymatic action of myeloperoxidase. Similar to glycation, carbamylation leads to profound modifications of proteins and in turn to drastic impairments of their biological functions. The main carbamylation-derived product, homocitrulline, can be detected in vivo and thus helps revealing such ongoing processes during aging and especially on elastin [204].

Moreover, during aging, elastin is calcified and interacts with various lipids, two interconnected processes. Calcification of elastin is a multistep process leading to the deposition of calcium phosphate minerals onto the elastic network. Ca\(^{2+}\) binding on elastin could involve either its interaction with carboxyl groups or uncharged β-turn structures permitting the recruitment of phosphate allowing the formation of hydroxyapatite [212,213]. Interestingly, it has been shown that damages present on the surface of elastin enhance mineral crystallization [214]. Ca\(^{2+}\) binding to elastin but also to EDPs leads to an increase of their affinity toward cholesterol, another deleterious biological process particularly observed during vascular aging [215]. Finally, it has been demonstrated that some elastin fragments present the ability to form amyloid-like structures that appear during aging and that could affect age-related pathologies [216,217]. Additionally, it has been elegantly shown that heparan sulfates facilitate harmless amyloidogenic fibril formation influencing cell adhesion [218].

As indicated earlier, besides these nonenzymatic processes, elastin suffers proteolytic degradation by several elastin-cleaving extracellular proteinases. The known elastases belong to three different families: cysteine proteinases cathepsins K, L, S, and V [219,220], matrix metalloproteinases (MMPs) with MMP-2, MMP-7, MMP-9, MMP-12, and MMP-14 [221–223], and serine proteinases with cathepsin G, proteinase 3, and neutrophil elastase [146,224,225]. These proteinases are primarily produced by fibroblasts, mesenchymal cells, platelets, macrophages, and leukocytes, and all of them exhibit elastolytic activity that can lead to a modification of elastin’s mechanical behavior. Besides detrimental mechanical consequences, elastin degradation causes the liberation of bioactive EDPs. The most studied elastokine is the VGVAPG peptide, but during the 10 past years, a plethora of other bioactive EDPs such as GVYPG, GFGPG, GVLPG have been discovered, and some longer bioactive EDPs such as YTTGKLPYGYGPQG, YGARPGVGVGP, and PGGFAVPGA have been shown to be released by the specified MMPs [83].

Strikingly, the above-mentioned aging-related processes are highly involved in the progression of several
serious pathologies, described in the next section, and in which elastokines play a central role (Fig. 3).

**Elastokines in metabolic and vascular diseases**

Elastin-derived peptides are powerful modulators of cell phenotype and they highly influence the progression of several metabolic and vascular diseases. It has been established that these peptides are critical regulators of the cardiovascular continuum [83] and could act at several levels ranging from the regulation of the cardiovascular risk factors to a drastic increase of pathologies-related processes. Interestingly, these peptides are able to drive insulin resistance and the appearance of characteristic features of type 2 diabetes [226]. Indeed, when injected into normal mice, EDPs drive an increase of body weight along with a decrease of glucose uptake by target organs leading to a hyperglycemia dependent on the presence of EDPs. The molecular mechanism involves the desialylation of the glycan chains of insulin receptor β-chain by the neuraminidase-1 subunit of the ERC, leading to a decrease in its signaling abilities and to the appearance of insulin resistance [227].

Elastin-derived peptides also exhibit a drastic influence on the development of nonalcoholic steatohepatitis [228]. Interestingly, it was shown that accumulation of EDPs triggers hepatic lipogenesis, fibrosis, and inflammation. The molecular pathways involved have been clearly identified and showed an inhibition of the LKB1-AMPK pathway following ERC activation. Recently, it was also shown that EDPs through the engagement of ERC decrease adipocyte differentiation via the modulation of the PPARγ transcription factor. Furthermore, the signaling pathway implicated in the EDPs-reduced cell differentiation effect has been described, involving an ERC/lactosylceramide/ERK1/2/PPARγ module, making it a pathway of interest in metabolic diseases associated with obesity [229].

Besides these involvements in metabolic diseases, EDPs have been described as central inducers of atherosclerosis. They have been shown to drive development of the early phase of the atheroma plaque in two mice models, ApoE<sup>−/−</sup> and LDLR<sup>−/−</sup>, in which EDPs were able to increase the formation of fatty streaks [189]. From a mechanistic point of view, these peptides have been demonstrated to trigger a Neu-1/PI3Kγ signaling module responsible for the recruitment of monocytes to the lesion site and amplifying their pro-oxidative activities. The use of chimeric mice moreover demonstrated the central role of the ERC. Recently, it was shown that besides these processes EDPs are able to increase oxLDL uptake by macrophages through the Neu-1-dependent desialylation of the CD36 receptor, reinforcing the central role in EDPs-driven atherosclerosis [230].

Remarkably, in addition to these deleterious effects, EDPs possess some beneficial effects on the cardiovascular system. It has been shown, for example, that they regulate platelet aggregation, as demonstrated by the study of Kawecki et al. [231]. This work showed that EDPs lower platelet aggregation triggered by several agonists, but also reduce the interactions between platelet and collagen under arterial shear conditions. Moreover, it has been shown that EDPs can trigger an endothelium-dependent vasodilatation through NO production, which could contribute to the control of the vascular tone during aging [232]. Finally, it has been shown that EDPs could exert cardioprotective effects during myocardial ischemia/reperfusion injury [233]. The authors showed that the presence of EDPs during reperfusion using the model of Langendorff has beneficial effects on left ventricular diastolic pressure, rate-pressure product, mean coronary flow, and myocardial infarct size. The molecular events leading to these protective effects implicated the EDP-mediated NO release and activation of the reperfusion injury salvage kinase pathway, suggesting that EDPs could act as powerful myocardial protectors in this context [233].

**Elastokines in tumor progression**

Aside from their crucial roles in metabolic and cardiovascular events, EDPs have been revealed to drastically contribute to cancer progression. One of the most striking characteristics is their ability to regulate both stromal and tumor cells phenotypes to promote cancer progression. One of their first described effects was the stimulation of protease expression by tumor cells participating to their migration and invasion properties [193,234–237]. Moreover, EDPs have been shown to present potent chemotactic activity on diverse cancer types such as melanoma cells that could contribute to the formation of a metastatic niche [238]. Besides these biological influences, EDPs also exhibit pro-proliferative effects in vitro, as demonstrated for glioblastoma [236,239], astrocytoma human cell lines [240], or murine melanoma cell line for which an in vivo pro-proliferative effect has also been documented [240].

The deleterious effect of EDPs on cancer progression also affect stromal cells as they are potent modulators of stromal reaction. For instance, they have been described to strongly modulate different processes affecting fibroblasts, inflammatory cells, or endothelial
Inherited diseases of elastin and microfibrils

Besides the contribution of elastin degradation in the above-mentioned pathologies, several genetic alterations of elastic fiber components have been described. In this context, genetic diseases associated with defects in elastin and microfibril proteins represent large groups of pathological conditions. It is, moreover, important to note that in some cases diseases affecting elastic fibers are due to mutations in genes not directly related to elastic fibers.

Inherited elastinopathies mainly arise from mutations in genes of elastin and elastic fiber-associated proteins leading to the onset of diseases characterized by an increased susceptibility to proteolytic cleavage or by a gain or loss of function. Among elastin-mutated gene diseases, supravalvular aortic stenosis (SVAS), the Williams-Beuren syndrome (WBS), or cutis laxa (CL) are the best-characterized phenotypes, along with pseudoxanthoma elasticum (PXE) that arises from ABCC6 or ENPP1 mutations [249–252].

SVAS is a disease with an incidence of 1 in 20 000 live births. It is an autosomal-dominant genetic alteration manifested by narrowing or obstruction of the ascending aorta that can also be observed in coronary, renal, carotid, or pulmonary arteries. In its severe forms, it can lead to congestive heart failure preceding by phenomena such as left ventricular hypertrophy or dyspnea [250]. Several genetic processes such as translocations, point mutations, or partial deletions affecting the elastin gene could lead to premature stop codons and unstable mRNA, and the accepted mechanism responsible for SVAS is elastin haploinsufficiency [253]. In this process, the SMCs produce only half of the required normal elastin level leading to thinner elastic fibers but also to hyperproliferation of SMCs [254].

SVAS is typically linked to WBS, a multifaceted developmental disorder linked to cardiovascular, craniofacial, neurocognitive, and metabolic abnormalities [249,253]. The prevalence of this rare genetic disorder is between 1 in 20 000 and 1 in 7500 live births. It is associated with glucose intolerance, hypocalcemia, and mitral valve dysfunction and caused by a microdeletion at the chromosome region 7q11.23, which encompasses up to 28 genes, including the elastin gene [255,256]. More rarely, WBS can result from duplication of the same regions but in this case, the observed phenotypes are milder [257]. Generally, one copy of the elastin gene is affected explaining why SVAS is associated with WBS, as WBS SMCs produce one-half of the normal levels of elastin [258].

PXE is an autosomal recessive genetic alteration with a prevalence of at least 1 in 56 000 live births [259] although the exact prevalence is subject to discussion ranging from 1 in 25 000 to 1 in 100 000 [251]. It is characterized by diverse cardiovascular, ocular, and cutaneous manifestations due to the mineralization and fragmentation of elastic fibers [252]. As cited above, it is mainly caused by mutations in the ABCC6 gene, encoding a transmembrane transporter highly expressed in the kidney and the liver [260]. Although the mechanisms linking the ABCC6 transporter to mineralization remain unclear, it has been clearly shown that there is a correlation between ABCC6 deficiency in animal models and PXE outcome. Moreover, it has been described that mutations in ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1) could also be involved, leading to reduced levels of circulating inorganic pyrophosphate (Pi), a calcification inhibitor, participating to the PXE phenotype [261,262].

CL comprises different inherited alterations characterized by loose, wrinkled skin that lacks elasticity and gives an elderly appearance [251]. CL has been described in autosomal recessive CL (ARCL) type-1 and 2, RIN2 syndrome, Urban-Rifkin-Davis syndrome, arterial tortuosity syndrome, Menkes disease, autosomal dominant CL (ADCL), and X-linked recessive CL (XLCL). ARCL type 1 results from mutations affecting fibulin-4 or fibulin-5 leading to aortic aneurysms or emphysema [263,264], whereas type 2 is due to a mutation in the proton pump gene ATP6V0A2 and affects intracellular vesicle trafficking [265]. RIN2 syndrome is caused by a mutation in the RIN2 guanine exchange factor, which also leads to an alteration of vesicular trafficking [266]. Urban-Rifkin-Davis syndrome is the result of mutations in LTBP4, causing developmental abnormalities, whereas arterial tortuosity syndrome, characterized by the appearance of tortuous arteries, is caused by a mutation in the gene encoding the GLUT10 transporter [267]. XLCL and Menkes diseases result from mutations in the ATP7A gene affecting elastin deposition [268]. Finally, ADCL,
which is the result of frameshift mutations at the 3’-end of the elastin gene, results in the translation of a TE mutant exhibiting an extended C-terminal missense peptide sequence, is a milder form of CL [269].

Acquired elastinopathies leading to alteration of elastic fiber function and/or structure have also been documented [269]. Such elastinopathies include, for example, the acquired forms of CL and PXE (due to dermal inflammation caused by reactions to drugs and to cutaneous mineralization) [270–272], and solar (actinic) elastosis. The latter is caused by cumulative effects of sun exposure and degeneration of the elastic tissue (photoaging) and is characterized by an accumulation of abnormal elastin in the dermis or the conjunctiva [273].

The most important group of fibrillinopathies arise from fibrillin-1 disorders. Fibrillin-1 is involved in the pathogenesis of complex genetic diseases such as Marfan syndrome (MFS; characterized by severe cardiovascular abnormalities like aortic dilation and aneurysms, arachnodactyly, loose skin, fragmented elastic fibers and increased TGFβ signaling) or MASS (mitral valve prolapse, aortic enlargement, skin, and skeletal findings) syndrome [7]. MFS is an autosomal dominant heritable genetic alteration observed with a prevalence of 1 in 5000 live births and arise from missense mutations, in-frame deletions, exon-skipping/splice-site mutations, and nonsense mutations leading to premature termination codons [274]. MASS syndrome is another disease resulting from mutations in the fibrillin-1 gene, presenting an overlapping phenotype with MFS. Two of the involved mutations have been described as frameshift mutations with premature termination of translation, one of which leads to decreased levels of the mutant transcript [275]. Fibrillin-2 and fibrillin-3 mutations are associated, respectively, with congenital contractural arachnodactyly (CCA; Beals syndrome) and polycystic ovary syndrome (PCOS) [276]. For CCA, an altered fibrillin-2 secretion has been described, although elastic fibers appear functional due to a partial rescue by fibrillin-1 [277]. PCOS is a common endocrinopathy affecting 6–10% of women worldwide [278] and, even if the mechanism remains elusive, a clear correlation between genetic alterations of fibrillin-3 gene, expression of fibrillin-3, and PCOS has been shown [276].

Besides these important groups of diseases, it has also been described that mutations in other elastic fibers proteins such as members of the LTBP family lead to several clinical manifestations such as Weill-Marchesani-like (WMS-like) syndrome characterized by cardiovascular, ocular, and joint defects [279]. In this particular disorder, for example, it has been shown that homozygous c.3529G>A (p. Val1177Met) mutations in the LTBP2 gene caused autosomal recessive WMS-like syndrome, leading to drastic alterations of elastic fibers and others ECM proteins [280].

**Conclusion**

Elastic fibers contribute significantly to the structural integrity and biomechanics of dynamic vertebrate tissues. Despite their vital importance for organ and tissue function, the structures and functioning of their main components, as well as many aspects of their formation and damage, are still not fully understood. This has to do in large part with elastin’s extraordinary properties, which have made the study of elastic fibers one of the most demanding endeavors in matrix biology.

However, significant advances in life sciences and bioinformatics in recent decades have enabled studies that have greatly expanded our understanding of gene expression and synthesis of the monomeric components of elastin and microfibrils, their assembly into functional elastic fibers, and important interactions during or after this process. It became also evident that the components of elastic fibers as well as their breakdown products, especially elastokines, are involved in the outcome and development of a large number of diseases. Much work, however, remains to be done to complete our understanding of the biology of the processes of elastogenesis and elastic fiber decay. This will contribute to the development of innovative therapies and advanced biomaterials for the treatment of severe pathological and age-related conditions.

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

CEHS and LD wrote the manuscript. CEHS prepared the figures.

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Formation and fate of elastic fibers

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