Collagen, stiffness, and adhesion: the evolutionary basis of vertebrate mechanobiology

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ABSTRACT The emergence of collagen I in vertebrates resulted in a dramatic increase in the stiffness of the extracellular environment, supporting long-range force propagation and the development of low-compliant tissues necessary for the development of vertebrate traits including pressurized circulation and renal filtration. Vertebrates have also evolved integrins that can bind to collagens, resulting in the generation of higher tension and more efficient force transmission in the extracellular matrix. The stiffer environment provides an opportunity for the vertebrates to create new structures such as the stress fibers, new cell types such as endothelial cells, new developmental processes such as neural crest delamination, and new tissue organizations such as the blood–brain barrier. Molecular players found only in vertebrates allow the modification of conserved mechanisms as well as the design of novel strategies that can better serve the physiological needs of the vertebrates. These innovations collectively contribute to novel morphogenetic behaviors and unprecedented increases in the complexities of tissue mechanics and functions.

INTRODUCTION

Three major driving forces in the evolution of vertebrates are the development of a stiffer extracellular environment, the emergence of collagen-binding integrins, and the significant increases in force generation in nonmuscle cells. These conditions, together with two rounds of whole genome duplications at the base of vertebrate evolution, give rise to new structures and cell types. The purpose of this Perspective is to speculate on how collagen I, stiffness, and adhesion impacted the evolution of mechanobiology, resulting in divergent mechanisms in vertebrates and nonvertebrates (Nakatani et al., 2007; Hufton et al., 2008; Hoffmann et al., 2012).

Multiple biological and animal models are used to study cellular behaviors such as tissue morphogenesis. However, in many instances, different systems produce conflicting observations and, in the field of mechanobiology, very different conclusions. These discrepancies are seen at many levels of investigations. At the tissue level, cell movement appears to result from different mechanisms during the development of Drosophila and Xenopus embryos (Keller, 1986; Keller et al., 1992; Keller and Winklbauer, 1992; Shih and Keller, 1992; Irvine and Wieschaus, 1994; Bertet et al., 2004; Zallen and Wieschaus, 2004; Shindo, 2018). At the signaling level, collective cell migration appears to be activated by distinct ERK-dependent mechanisms in Drosophila and mammalian cells (Aoki et al., 2017; Ogura et al., 2018). At the molecular level, different amounts of molecular force appear to be exerted at cell–cell adhesions in the developing Drosophila and zebrafish embryos (Yamashita et al., 2016; Eder et al., 2017; Lagendijk et al., 2017). Furthermore, the physiological roles of mechanosensitive molecules such as vinculin also appear to be different. For example, knockout of vinculin has little effect in Drosophila but is detrimental to zebrafish and mice (Alatortsev et al., 1997; Xu et al., 1998; Zemljic-Harpf et al., 2004; Cheng et al., 2016; Lausecker et al., 2018). In Drosophila, hyperactive vinculin inhibits the formation of integrin adhesion complexes, whereas the opposite is true for the mouse (Marg et al., 2010; Maartens et al., 2016). At the cellular level, novel cellular and molecular structures are found only in vertebrates. One novel structure found in nonmuscle cells is the stress fiber, which has an alternating actin and myosin II arrangement, analogous to the sarcomeres in muscle cells. The “sarcomere-like”...
Collagen I is the primary component of the vertebrate extracellular matrix (ECM), making up ~30% of the total protein mass in humans (Huxley-Jones et al., 2007; Kadler et al., 2007; Silver, 2009; Exposito et al., 2010). Collagen I forms thick, stiff, and long fibrils that are responsible for the dramatic decrease in tissue compliance in vertebrates (Silver et al., 2001, 2002; Ushiki, 2002; Boot-Handford and Tuckwell, 2003; Yang et al., 2008). Depending on the density and packing order of collagen I fibrils, vertebrates can construct ECM up to hundreds of megapascals in stiffness (van der Rijt et al., 2006; Candiello et al., 2007; Shen et al., 2008, 2011; Yang et al., 2008; et al., 2010; Kubow et al., 2015; Lemmon and Weinberg, 2017; Mezzenga and Mitsu, 2019). Thus, the emergence of collagen I not only increases the stiffness of the overall extracellular environment, it also alters the rigidity and organization of the fibronectin network, allowing a softer meshwork-type matrix to behave as a stiffer fibrillar-type substrate. This has far-reaching implications and consequences because fibronectin-binding integrins have mechanotransduction roles and can sense the stiffness of the fibronectin matrix (Tee et al., 2011; Trichet et al., 2012; Carraher and Schwarzbauer, 2013; Ribeiro et al., 2014; van Geemen et al., 2015). Thus, the emergence of collagen I supports a wider range of mechanotransduction responses elicited by fibronectin-binding integrins. In vertebrates, fibronectin can assemble with collagen I to form fibers that potentially can interact with both fibronectin-binding and collagen-binding integrins concomitantly (Kadler et al., 2008; Singh et al., 2009; Carracedo et al., 2010; Hu et al., 2011; Niland et al., 2011; Popov et al., 2011; Svensson et al., 2010; Aifantis et al., 2011; Kohn et al., 2015; Mineralization of collagen I fibers further increases the stiffness of the matrix to several gigapascals, a magnitude that is orders of magnitude higher than the ECMs than most nonvertebrates would have ever experienced (Figure 1A) (Kawahara et al., 2004; Landis and Silver, 2009; Chlasta et al., 2017).

THICKER AND STIFFER FIBRILS INCREASE THE STIFFNESS OF EXTRACELLULAR MATRIX

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CONNECTING ECM TO CELLS

The major force-transmission pathway connecting the force-generating actomyosin structures inside the cells to the ECM occurs via cell surface integrins (Figure 1B) (Niland et al., 2011; Livne and Geiger, 2016; Carey et al., 2017). Vertebrate and nonvertebrate cells can interact with the evolutionarily more conserved fibronectin and laminin through fibronectin-binding and laminin-binding integrins. However, only vertebrates have evolved collagen-binding integrins to interact with collagens. Thus, despite the presence of collagen IV in all metazoa, nonvertebrate cells do not interact directly with collagen IV because they do not have the collagen-binding integrins (Ewan et al., 2005; Chouhan et al., 2014; Johnson and Chouhan, 2014).

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FIGURE 1: A reductionist’s view of the extracellular experience in vertebrates and nonvertebrates. (A) The evolutionarily conserved fibronectin, collagen IV, and laminin form meshwork-type matrices with stiffnesses up to hundreds of kilopascals (kPa). In vertebrates, collagen I forms matrices with stiffnesses ranging from megapascals (MPa) to gigapascals (GPa). Nonvertebrates and vertebrates can bind to laminin and fibronectin, whereas only vertebrates interact directly with collagen (see the text for details). (B) Two cells are interacting with the ECM and with each other. Force generated by stress fibers inside the cell can be transmitted to the extracellular collagen matrix via collagen-binding integrins. A basement membrane containing a mixture of matrix proteins is present in some specialized tissues.
Zeltz and Gullberg, 2016; Ciuba et al., 2018). The direct coupling between integrin and collagen plays a significant role in the generation of higher tension and more efficient force transmission in the collagen matrix (Hu et al., 2011; Mohammadi et al., 2015b). Furthermore, the ability to exert force directly on collagen fibers allows the acquisition of novel cellular behaviors including multicellular streaming and contact guidance (Tiger et al., 2001; Tamariz and Grinnell, 2002; Wolf et al., 2003, 2009; Miron-Mendoza et al., 2010; Carey et al., 2016; Grossman et al., 2016; Han et al., 2016; Nuhn et al., 2018; Sarker et al., 2019). Collagen-binding integrins and fibronectin-binding integrins form discrete molecular complexes and have distinct mechanotransduction functions, contributing to cell-matrix biology in complementary ways in vertebrates (Ylanne et al., 1993; Hocking et al., 2000; Teravainen et al., 2013; Ribeiro et al., 2014; Burridge and Guilluy, 2016; Roca-Cusachs et al., 2013).

**COLLAGEN I SUPPORTS LONG-RANGE FORCE TRANSMISSION**

Vertebrate collagen I has evolved new biochemical strategies to form compact, staggered, and covalently cross-linked fibrils that are thicker, stiffer, and longer than any ancestral fibrillar or meshwork collagens (Pins et al., 1997; Eyre and Wu, 2005; Ricard-Blum, 2011; Kwansa et al., 2016). Collagen I is more efficient for directional force propagation due to the increased fiber length, the higher rigidity, and the precise alignment of collagen I molecules in the fibers. The amount of force that can be generated in collagen I matrices is substantially higher, up to several orders of magnitude, than that that can be supported by fibronectin, laminin, or collagen IV meshwork-type matrices (Hocking et al., 2000; Boot-Handford and Tuckwell, 2003; Candiello et al., 2007; Dominguez-Gimenez et al., 2007; Araki et al., 2009; Silver, 2009; Exposito et al., 2010; Maruthamuthu et al., 2011; Sztal et al., 2011; Tee et al., 2011; Fahey and Degnan, 2012; Trichet et al., 2012; Roca-Cusachs et al., 2013; Teravainen et al., 2013; Cetera et al., 2014; Xie et al., 2014; Adams et al., 2015; Kubow et al., 2015; Burridge and Guilluy, 2016; Goodwin et al., 2016; Chlasta et al., 2017; Fidler et al., 2017, 2018; Filla et al., 2017; Qin et al., 2017; Sancho et al., 2017; Shook et al., 2018; Zollinger et al., 2018; Draper et al., 2019; Cerqueira Campos et al., 2020; Chronopoulos et al., 2020).

How exactly might collagen I bring new mechanobiology to vertebrates? At least four factors are important (Figure 2):

1. **The number of binding sites matters.** The number of integrin-binding sites on a matrix molecule determines whether it can cluster the integrins and how much force it can transmit (Figure 2A). A laminin trimer interacts with only one integrin, whereas a fibronectin dimer has two integrin-binding sites. A collagen fibril contains tens to hundreds of covalently cross-linked molecules, providing many integrin-binding sites (Xu et al., 2000; Pichard et al., 2001; Turner et al., 2020). In vivo, fibronectin can form larger assemblies via noncovalent interactions, increasing the apparent binding sites. Collagen IV can also form large assemblies via noncovalent interactions and disulfide bonds. However, these networks are deformable and can buckle, collapse, or rupture when stretched. On the other hand, the extensively cross-linked collagen fiber behaves as one unit under force. Thus, the total amount of force that can be applied to a collagen fiber is substantially higher than the forces that can be applied to meshwork-type matrices. Multiple interactions between integrins and the collagen fiber would allow force integration necessary for stiffness sensing by the cells (Jiang et al., 2016; Janmey et al., 2020).

2. **Size and length matters.** The nonmuscle myosin II minifilament, which is ~300 nm long, forms the force-generating unit of the stress fiber (Pellegrin and Mellor, 2007; Billington et al., 2013; Dasbiswas et al., 2018). To generate tension at integrin adhesions, two integrins must be positioned at least as far apart as the length of a myosin II minifilament. A fibronectin dimer has a...
length of ∼100 nm, and while it can cluster two integrins it cannot support tension generation. Thus, contraction of the stress fiber will pull on both integrins, dragging the fibronectin dimers and the integrins in the same direction. If the fibronectin dimers are isolated, there will be no resistance to the movement and no tension will be generated within the matrix (Figure 2B). In a fibronectin network, pulling on fibronectin would lead to stretching of the network. However, if the pulling force exceeds the strength of the noncovalent bonds, the fibronectin network could break, interrupting the force-transmission pathway and tension generation. Collagen IV meshworks are held together by disulfide bonds and noncovalent interactions and thus can withstand pulling force when stretched, possibly permitting tension to build up in the distorted matrix (Figure 2C). In vertebrates, a collagen I fiber can be up to tens of microns in length and thus can integrate many “sarcomere-like” contractile units, increasing the force exerted at individual cell-matrix adhesions and resulting in greater tension to be generated within the ECM. When two noncontacting cells are bound to the same long collagen I fiber, pulling force from one cell can be sensed by a neighboring cell (Figure 2D). Traction force generated at collagen-binding integrins can realign or translocate the collagen I fiber, resulting in the remodeling of the ECM (Ramos and DeSimone, 1996; Davidson et al., 2002, 2004, 2008; Marsden and DeSimone, 2003; Miron-Mendoza et al., 2008; Wang et al., 2014; Han et al., 2018; Ban et al., 2019; Shifflett et al., 2019).

(3) Stiffness matters. Matrixes composed of fibronectin, laminin, and collagen IV form meshworks that are stretchable by cellular force, whereas collagen I is long and compact fibers that are stiff and relatively unbendable by cellular force. The propagation of force within fibronectin and collagen IV meshworks primarily goes through noncovalent protein–protein interfaces. In the presence of pulling force that can break noncovalent bonds, meshwork-type matrixes will fail to transmit force while the collagen cross-linked collagen I fiber will continue to support force propagation. In vertebrates, the interaction of fibronectin with fibrillar collagen I via noncovalent bonds could alter the apparent biophysical properties and stiffness of the fibronectin network. However, fibronectin is extremely distensible and unfolds almost completely under physiological force, reaching a tensile stiffness of only 1–2 MPa when maximally stretched, which is at least two orders of magnitude lower than the tensile stiffness of collagen I fibrils (van der Rijt et al., 2006; Shen et al., 2008; Yang et al., 2008; Klotzsch et al., 2009; Svensson et al., 2010; Bradshaw and Smith, 2014). Moreover, fibronectin–integrin bonds are not as strong as collagen–integrin bonds. Therefore, force propagation in collagen I matrix is much more efficient due to the covalent intermolecular bonds, the higher tensile stiffness of the collagen I fibers, and the stronger bonds between collagen and collagen-binding integrins. In this regime, higher levels of tension can be accumulated within the ECM. The tensile energy in the stiffener matrix, in turn, would increase the stiffness of the cell and promote greater force generation that can be used to assemble stress fibers, remodel focal adhesions, promote cell–cell adhesions, and ultimately increase the overall potential energy of the tissue (Walcott and Sun, 2010; Maruthamuthu et al., 2011; Wolfenson et al., 2011; DeMali et al., 2014; Wang et al., 2014; Wong et al., 2014; Ye et al., 2014; Broaders et al., 2015; Ronan et al., 2015; Hall et al., 2016; Hu et al., 2017; Le et al., 2017; Dasbiswas et al., 2018; Han et al., 2018; Kuragano et al., 2018b; Chang et al., 2019).

(4) Geometry matters. Collagen IV and fibronectin form meshworks that are isotropic and would dissipate force in all directions, thus cannot faithfully reproduce the vector quality of force (Figure 2A). In contrast, collagen I fibrils behave like linear cables that can support the propagation of directional information, alignment of stress fibers, and the asymmetric growth of focal adhesions (Figure 2D). Importantly, linear collagen I fibers provide physical cues for directed protrusions and cell migration (Kaunas et al., 2005; Besser and Safran, 2006; Miron-Mendoza et al., 2010; Foonlen et al., 2014; Tondon and Kaunas, 2014; Mohammadi et al., 2015a; Han et al., 2016; Xie et al., 2017; Brauer et al., 2019; Sarker et al., 2019).

THICKER, STIFFER, LONGER FIBRILS SUPPORT HIGHER FORCE PRODUCTION

Vertebrate cells stiffen when attached to collagen I, exerting forces up to hundreds of nanonewtons which is nearly an order of magnitude higher than that can be generated by fibronectin-attached cells (Hocking et al., 2000; Araki et al., 2009; Maruthamuthu et al., 2011; Tee et al., 2011; Trichet et al., 2012; Roca-Cusachs et al., 2013; Teravainen et al., 2013; Kubow et al., 2015; Burridge and Guillev, 2016; Sancho et al., 2017; Zollinger et al., 2018; Chronopoulos et al., 2020). Experimental evidence indicates that the maturation process of fibronectin-based adhesions is independent of force (Oakes et al., 2012; Stricker et al., 2013), raising the possibility that fibronectin-based adhesions are fundamentally different from collagen-based adhesions.

There are many possible ways to increase force production at collagen-based adhesions in vertebrates:

(1) Turn on myosin II ATPase. One way to increase force production is to activate pathways that phosphorylate and activate myosin II and turn off pathways that dephosphorylate and inactivate myosin II (Tójkander et al., 2012; Ciuba et al., 2018). The molecular motor myosin II is responsible for force generation in cells and is regulated by the phosphorylation status of the myosin light chain. In cells, myosin light chain is phosphorylated by myosin kinase. Thus, activating signals such as Rh, Rock, and calcium-calmodulin that increase the activities of myosin kinase would stimulate myosin contraction. Myosin phosphatase, in turn, dephosphorylates the myosin regulatory light chain and ends contraction. Activating signaling pathways that inhibit myosin phosphatase will promote myosin light chain phosphorylation and sustained contractility.

(2) Modify core machinery. To increase force production, myosin II assemblies with more motor heads can be formed by regulating the stability and the assembly of myosin II minifilaments. Increasing the duty ratio of the myosin motor would increase the processivity of individual motors. In addition, altering the mechanical properties of the myosin motor would increase the strength of the myosin–actin bond under mechanical stress. These properties are already implemented by the vertebrate-specific myosin IIB (Figure 3A), a parologue of the ancestrally derived myosin IIA (Murakami et al., 1998, 2000; Chantler et al., 2010; Stam et al., 2015; Heissler and Sellers, 2016; Kuragano et al., 2018a; Melli et al., 2018).

(3) Build new structures using existing components. Each myosin II minifilament is capable of producing force of ∼30–50 piconewtons, but when the cells are attached to the collagen I matrix, they can generate forces up to hundreds of nanonewtons (Kaya and Higuchi, 2010; Sim et al., 2015; Sancho et al., 2017).
This means that thousands of myosin II minifilaments must work synergistically to produce the amount of force that is measured at collagen I–based adhesions. To amplify force production, the cell organizes the myosin minifilaments and arranges them in parallel and in series, rather than randomly positioning them inside the cytoplasm (Figure 3B). Indeed, this organization is used in vertebrate nonmuscle cells to build actomyosin stress fibers capable of generating force that is an order of magnitude higher than the sum produced by individual myosin motors (Pellegrin and Mellor, 2007; Lohner et al., 2019).

Forces generated by stress fibers are transmitted to the ECM through focal adhesions, which are sites of primary traction consisting of clustered integrins and strengthened linkage to actin (Ye et al., 2014; Burridge and Guilloy, 2016; Livne and Geiger, 2016). Focal adhesions formed by collagen-binding integrins are essential for vertebrate mechanobiology by participating in stiffness sensing, cell migration, and remodeling of the ECM (Burridge and Fath, 1989; Fath et al., 1989; Plotnikov et al., 2012; Bays et al., 2014; Hall et al., 2016; Chang et al., 2019; Puleo et al., 2019).

TUNING FORCE IN A STIFFER ENVIRONMENT SUPPORTED BY WHOLE GENOME DUPLICATIONS

To manage the new biology that comes with a stiffer environment, vertebrates are provided with a new toolbox, brought in part by a major evolutionary event consisting of two rounds of whole genome duplications (Vandepoele et al., 2004; Dehal and Boore, 2005; Holland and Ocampo Daza, 2018; Marletaz et al., 2018). This major evolutionary event gives rise to paralogues at a genomic scale that marks the branching of vertebrates from the invertebrates ~500–550 million years ago. Thus, a significant percentage of adhesion and cytoskeletal proteins in vertebrates have multiple copies, one from the ancestral gene and the others from duplicated genes, for example, filamin A-C, paxillin α-γ, myosin IIA-C, α-actinin-1-4, tropomyosin-1-4, WAVE-1-3, RhoA-C, Rac1-3, and talin1-2 (Boureux et al., 2007; Gehler et al., 2009; Kurisu and Takenawa, 2009; Rahimzadeh et al., 2011; Billington et al., 2013; Austen et al., 2015; Mohammadi et al., 2015b; Meacci et al., 2016; Schifflauer et al., 2016; Kuragano et al., 2018a; Pathan-Chhatbar et al., 2018; Puleo et al., 2019; Sao et al., 2019). While the ancestral copy often retains its original function, the duplicated copies frequently acquire new functions due to the lack of selection pressures on the copies. The expanded protein toolbox provides new options for the vertebrates to increase complexity and evolve novel molecular structures, mechanisms, and regulations (Brady et al., 2009; Laurin et al., 2019).

One way to build complexity into mechanoregulation is to adjust force production and tune mechanoresponses. Vertebrates have many options to handle this challenge:

1) **Assign new functions to an ancestral protein that has no essential function in nonvertebrates.** For example, vinculin is expendable in Drosophila but, in vertebrates, it plays an important role in the strengthening of cell–cell and cell–matrix adhesions under mechanical stress (Alatortsev et al., 1997; Cheng et al., 2016; Bays and DeMali, 2017; Le et al., 2019).

2) **Evolve new proteins and structures that are absent in nonvertebrates.** Many newly emerged vertebrate proteins and structures are designed to work with conserved molecules and core mechanisms to increase complexity (Mariani et al., 2020). One example is the intermediate filament vimentin, which localizes to focal adhesions and regulates the assembly of integrin adhesion complexes under mechanical stress (Tsutita and Jones, 2003; Sanghvi-Shah and Weber, 2017). Another example is the tight junction, which can modulate the mechanical input at the adherens junction in epithelial cells (Hatte et al., 2018).

3) **Subfunctionalize paralogues to tune force production.** Force generation can be adjusted by controlling stress fiber dynamics or focal adhesion stability. One example of subfunctionalization is the differential use of SORBS family members to control the amount of force applied to the ECM (Kuroda et al., 2018). While the vertebrate paralogue SORBS2 interacts with α-actinin to regulate stress fiber contractility, the ancestral paralogue SORBS1 interacts with vinculin to control focal adhesion maturation (Ichikawa et al., 2017). Another example is the differential usage of myosin IIA and IIB paralogues, resulting in the spatial regulation of stress fiber formation, front–back polarity, ECM remodeling, and intercellular junction dynamics (Even-Faitelson and Ravid, 2006; Vicente-Manzanares et al., 2007; Sandquist and Means, 2008; Solinet and Vitale, 2008; Smutny et al., 2010; Doyle et al., 2012; Gutzman et al., 2015; Stam et al., 2015; Ridge et al., 2017; Kim et al., 2018; Kuragano et al., 2018a,b; Heuze et al., 2019).

A COMPLEX EXPERIENCE FOR THE VERTEBRATE CELL

The expanded toolbox, along with collagen I and collagen-binding integrins, created exceptional opportunities for the vertebrates to build complexities in mechanobiology (Figure 4, A and B). To illustrate, two cells were drawn in Figure 4A; the cell on the left has a myosin II minifilament that is composed of myosin IIA (red motor heads) and IIB (black motor heads). The hybrid myosin IIA/IIB minifilament is attached to two focal adhesions containing collagen-binding integrins; one focal adhesion is bound to collagen IV monomers

myosin II filament

mechanosensitive Catch Bonds

higher Duty Ratio

Regulation of Filament Assembly

increase Force Generation

increase Processivity

amplification of Force Production by arranging myosin II

in Parallel & in Series

FIGURE 3: Controlling force production by myosin II regulation. (A) Force generation by stress fibers can be modified by increasing the duty ratio of the myosin II motor, processivity of the myosin II minifilament, mechanosensitivity of the myosin molecule, and the size of the myosin minifilament. (B) Force production by myosin II minifilaments can be amplified by arranging them in series and in parallel.
meshwork (red dotted circle on the left cell), and the other focal adhesion is bound to a collagen I fiber. Myosin IIB pulls harder than myosin IIA and moves the minifilament toward the collagen I fiber, exerting force on the collagen IV meshwork and ultimately breaking the noncovalent bonds within the collagen IV matrix (Figure 4B, red dotted oval on the left cell). Using the same argument, the cell on the right in Figure 4A has a hybrid myosin II minifilament composed of myosin IIA (red motor heads) and IIB (black motor heads). The stress fiber is attached to two focal adhesions; one focal adhesion is bound to the fibronectin meshwork via fibronectin-binding integrins (red dotted circle on the right cell), and the other focal adhesion is bound to a collagen I fiber via collagen-binding integrins. (B) Contraction force from the stress fibers can differentially regulate cell-matrix adhesions and extracellular force transmission in the matrix. In the cell on the left, myosin IIB pulls harder than myosin IIA and moves the minifilament toward the collagen I fiber, exerting force on the collagen IV meshwork and ultimately breaking the noncovalent bonds within the collagen IV matrix (red dotted oval on the left cell). In the cell on the right, myosin IIB wins the tug-of-war and the myosin IIA/IIB minifilament moves toward the collagen I fiber, pulling on the fibronectin-binding integrins and breaking their interaction with the fibronectin matrix (red dotted oval on the right cell).

**CONCLUDING REMARKS**

The emergence of collagen I drastically changed the compliance, porosity, stiffness, and organization of the ECM in vertebrate tissues. However, changing the biophysical properties of the extracellular environment alone is not sufficient for the evolution of new processes unless the stiffer matrix is linked to the cell. This is achieved by the coemergence of collagen-binding integrins at the base of the vertebrate evolution ~550 million years ago, resulting in a powerful change in the energy landscape in cells and in tissues. Ultimately, the dawn of vertebrate mechanobiology is realized by two rounds of whole genome duplications, providing new tools for regulation, amplification, and integration.

The decrease in tissue compliance allows the development of blood pressures to support pressurized renal filtration, a vertebrate invention that is superior in design for the management of electrolytes and metabolites (Schulte et al., 2015; Stephenson et al., 2017; Soslau, 2020). The increase in hydrostatic pressures in the closed circulatory system of the vertebrates necessitates the development of a vascular lining to prevent transmural leakage of vascular contents. The emergence of endothelial cells that line the vascular wall
is another significant vertebrate innovation that co-emerged with collagen I and collagen-binding integrins. Endothelial cells are equipped with specialized cell–cell junctions to act as permeability barriers and have strengthened cell–cell adhesions to resist hydraulic mechanical stress. The plasticity of the endothelial cells allows functional specialization of the endothelium, including a system of low-pressure capillary beds for nutrient delivery and the formation of the blood–brain barrier (Bundgaard and Abbott, 2008; O’Brien et al., 2018). The morphogenesis and the development of the endothelial vasculature, a process known as angiogenesis, is dependent on collagen-binding integrins on the endothelial cells and is supported by the collagen I ECM (Senger et al., 1997; Sweeney et al., 2003; Whelan and Senger, 2003; San Antonio et al., 2009; Kick et al., 2016; Post et al., 2019; Turner et al., 2020).

The endothelium ushers the appearance of multiple vertebrate-specific organs that are the footprints and signatures of vertebrate evolution (Shigeki et al., 2001; Vize and Smith, 2004; Munoz-Chapuli et al., 2005; Monahan-Earley et al., 2013). Endothelial cells are essential for the development of the vertebrate liver, pancreas, lungs, and kidneys during embryo morphogenesis. In adult animals, endothelial cells play significant roles in the physiological functions of the kidneys to concentrate urine and the lungs to perform gaseous exchange (Lammert et al., 2003; Cleaver and Dor, 2012; Ramsamy et al., 2015; Rafii et al., 2016; Bastidas-Ponce et al., 2017; Daniel and Cleaver, 2019). The morphogenesis of these vertebrate-specific organs is dependent on the proper deposition and organization of collagen I (Aycocock and Seyer, 1989; Goldstein, 1991; Saelman et al., 1995; Shimeld and Holland, 2000; Chen et al., 2004; Riopel and Wang, 2014; Buchler et al., 2018; Stephens et al., 2018).

Endothelial cells also play a significant role in the development of neural crest derivatives including the vertebrate skull, the brachial skeleton, and the sensory ganglia. Endothelial cells interact with neural crest cells, another key innovation of the vertebrates, and influence the distribution, the migratory pathways, and the patterned formation of neural crest cell derivatives. Neural crest migration from their origin of formation between the neural plate and the surface ectoderm is dependent on collagen I and requires interaction with the matrix via collagen-binding integrins (Duband and Thiery, 1987; Perris et al., 1991; Nagy et al., 2009; Diogo et al., 2015; George et al., 2016).

Further along the evolutionary timeline, mammals continue to utilize fibrillar collagens and collagen-binding integrins to invent new mechanosensitive processes, structures, and physiology, including the development of the mammalian placenta and invasion of the uterine wall by trophoblasts in a stiffness-dependent manner during embryo implantation (Damsky et al., 1994; Braasch et al., 2009; Yoshida et al., 2014; Griffith and Wagner, 2017; Park et al., 2017; Abbas et al., 2019; Zambuto et al., 2019). In conclusion, vertebrate mechanobiology is created, in part, by three major evolutionary forces including collagen I, collagen-binding integrins, and vertebrate-specific proteins to support the development of novel molecular mechanisms, adding to the repertoire of mechanisms that are already in place and shared by nonvertebrates and vertebrates (Rozario and DeSimone, 2010).

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