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INVITED REVIEW

Mechanotransduction: the role of mechanical stress, myocyte shape, and cytoskeletal architecture on cardiac function

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Abstract Mechanotransduction refers to the conversion of mechanical forces into biochemical or electrical signals that initiate structural and functional remodeling in cells and tissues. The heart is a kinetic organ whose form changes considerably during development and disease, requiring cardiac myocytes to be mechanically durable and capable of fusing a variety of environmental signals on different time scales. During physiological growth, myocytes adaptively remodel to mechanical loads. Pathological stimuli can induce maladaptive remodeling. In both of these conditions, the cytoskeleton plays a pivotal role in both sensing mechanical stress and mediating structural remodeling and functional responses within the myocyte.

Keywords Mechanotransduction · Heart · Cytoskeleton · Cardiac sarcomere · Cardiac myocytes · Mechanosensitivity

Introduction

Mechanotransduction is the process by which cells transduce mechanical forces to chemical and electrical responses. In the heart, mechanotransduction is necessary for balancing cell and tissue structure and function. Protein complexes that sense mechanical stimuli couple to the cytoskeleton, distinguishing it as an integrator sensitive to perturbations from extracellular, intracellular, and intercellular stimuli. Furthermore, because mechanosensing proteins associate with specific cytoskeletal substructures, such as the Z-disc, the architecture of the cytoskeleton endows myocytes with signaling biases and filters that attenuate mechanosensitivity and mechanotransduction based on the direction, source, and temporal frequency of mechanical inputs. One of many responses triggered by mechanical stimulation is cytoskeletal reorganization, closing a feedback loop between mechanosensitivity and structural remodeling as myocytes attempt to reach homeostasis. The cytoskeletal scaffold is also a network which propagates mechanical signals throughout the myocyte, triggering a wide range of functional responses.

With this review, we first describe in vivo correlations between myocyte shape and stages of development and disease, which have motivated the hypothesis that the cytoskeleton balances acute and chronic biomechanical stimuli by remodeling myocyte form and function. Next, we describe some of the mechanosensing proteins associated with myofibrils to demonstrate that the cytoskeletal network serves as a mechanosensory integrator. We then focus on in vitro studies that replicate the cardiac cellular microenvironment to elucidate how mechanosignaling regulates cytoskeletal remodeling. We argue that cytoskeletal architecture integrates mechanical inputs and regulates a wide range of mechanotransductive outputs or physiological responses to form a feedback loop for maintaining cardiac function in response to microenvironmental perturbations (Fig. 1).

Cell shape in the developing and diseased heart

The organization of the heart across multiple spatial scales, from the alignment of individual sarcomeres to gross

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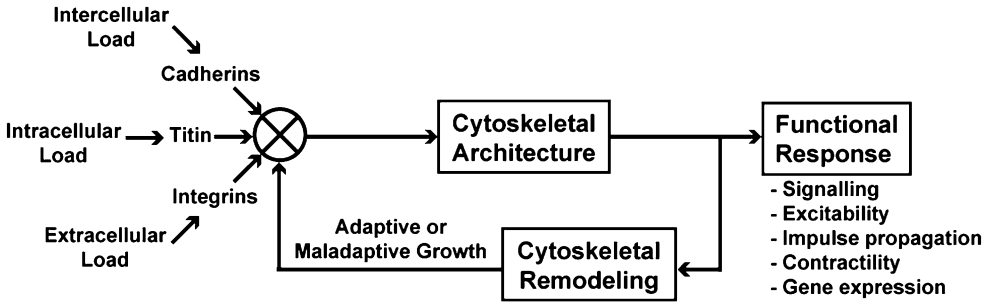

Fig. 1 Cardiac myocytes integrate intercellular, intracellular, and extracellular loads, which are sensed by many protein structures associated with the cytoskeleton, such as cadherins, titin, and integrins, respectively. The cytoskeleton remodels its architecture with adaptive or maladaptive growth in response to a variety of stimuli, including mechanical forces or biochemical factors. The cytoskeleton also propagates mechanical inputs and mediates functional responses, such as signaling, excitability, impulse propagation, contractility, and gene expression. Together, this feedback loop maintains homeostasis in a dynamic mechanical environment

chamber morphology, has important physiological implications. In adult ventricular tissue, cardiac myocytes are elongated and aligned along a common axis, facilitating rapid electrical propagation and the uniaxial alignment of sarcomeres, both of which contribute to spatially and temporally ordered contraction [136]. Lessons from in vivo studies hint that the emergence of cardiac form is in response to mechanical stimuli. This feedback potentiates chamber formation in the developing heart [166], but can lead to pathological remodeling [73] when an equilibrium condition is unattainable. Here, we detail in vivo studies of physiological and pathological growth that identify the cytoskeleton as the primary mediator between external stimuli and intrinsic remodeling of cell architecture.

Myocyte shape and physiological development

In the embryo, the linear heart tube comprises an outer layer of epithelial myocardium and an inner layer of endocardium separated by cardiac jelly. During cardiac looping, the heart tube asymmetrically bends and twists to the right as one of the first steps towards forming a complex, four-chambered heart [60, 128, 180]. Although looping is regulated by many factors, including local differences in gene expression [43, 165], proliferation [162, 178], and differentiation [48], evidence suggests that active changes in myocyte shape contribute significantly [144, 167]. Before looping, the myocardium of the outer heart tube layer is a homogeneous layer of columnar epithelial tissue [122]. Looping occurs due to asymmetric changes in the muscular layer, where myocytes in localized regions flatten, resulting in thinning of one side of the tube. After looping, myocardium on the concave side remains thick and columnar, versus the flatter convex side [122]. Although these regional changes in cell shape could be a passive response to extrinsic forces, heart tubes bend even when removed from the embryo [124], indicating that looping is caused by a local, intrinsic mechanism, which was later found to be actin filament growth [98, 102, 112, 123, 146]. Together, these studies suggest that actin dynamics actively induce heart tube looping by flattening cells exclusively on the convex side, illustrating how localized remodeling of cell shape contributes to the emergence of gross morphological form.

Although myofibril contractility is not considered a direct cause of looping [123], it does constrain myocyte shape in the ventricle, as demonstrated by mutant zebrafish hearts with severely compromised ventricular contractility [7]. Ventricular myocytes in these mutant hearts elongate relative to control hearts (Fig. 2a, b) [7], possibly because myofibrils normally sustain cellular tension and form [100]. Additionally, the entire ventricle in these mutants is distended (Fig. 2a, b), suggesting that microscale changes in myocyte shape caused by disruption of cytoskeletal tension impact gross morphology of the heart [7].

Local cell shape changes in the heart tube are thought to be regulated by biomechanical forces, such as hemodynamic pressure. Developing zebrafish hearts doped with glass microbeads to occlude blood flow into the ventricle fail to loop [95], illustrating a direct link between hemodynamic pressure and cardiac morphogenesis. This study also revealed that blood forms vortices inside the ventricle, creating gradients of sheer stress that could potentially signal localized differences in cellular and cytoskeletal remodeling [95]. In a complementary study, mutant zebrafish hearts with inefficient blood flow into the ventricle due to loss of atrial sarcomeres have smaller ventricles [15] consisting of cuboidal myocytes [7], again indicating that hemodynamic pressure regulates both ventricular myocyte morphology and chamber size. These studies suggest that localized cytoskeletal remodeling occurs to counterbalance extrinsic hemodynamic pressure in the developing heart.

Cardiac development is also regulated by the extracellular matrix (ECM), which forms a mesh of structural and signaling networks encapsulating and connecting the cells

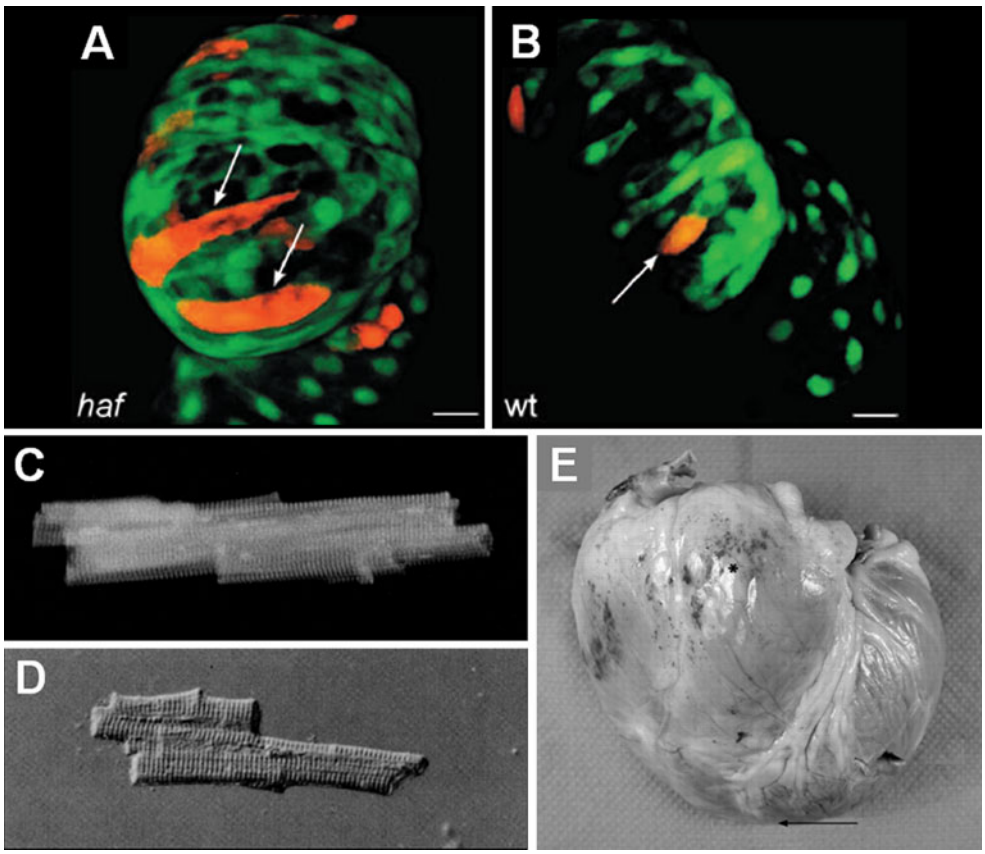

Fig. 2 Changes in myocyte shape during development and disease. Cardiac myocytes in *haf* zebrafish mutants cannot contract due to a genetic mutation in ventricular myosin heavy chain. Consequently, myocytes are larger in size and have a higher length to width aspect ratio (**a**) than myocytes in wild-type hearts (**b**), suggesting that intrinsic mechanical forces regulate cell shape and chamber morphology by maintaining cellular tension. Both hearts expressed *Tg*(*cmlc2:egfp*) with mosaic expression of *Tg*(*cmlc2:dsredt4*) to better visualize individual myocytes. Modified from [7]. Similar myocyte shape changes are observed in patients with ischemic cardiomyopathy, which is associated with increases in myocyte length (**c**) compared to myocytes from healthy hearts (**d**). Modified from [76, 118]. This change in myocyte shape is thought to underlie dilation of the ventricle (**e**), suggesting pathological stimuli induce changes in myocyte shape that dictate contractile function and whole heart morphology. Reproduced from [118] with permission from BMJ Publishing Group Ltd.

of the heart. Communication between the ECM and myocytes is facilitated by transmembrane integrin receptors that attach extracellularly to ECM ligands and intracellularly to the actomyosin cytoskeleton via proteins such as talin, vinculin, and α-actinin [10, 96, 148]. Each integrin receptor is a dimer consisting of single α and β subunits, and different combinations of these subunits endow the receptor with specificity for ECM ligands [97]. For example, α5β1 integrins are expressed on the surface of cardiac myocytes and specifically bind fibronectin [148]. In addition to providing structural support by tethering cells to the ECM, integrins also regulate many processes within the cell, such as migration [10, 147], proliferation, and signaling [126, 154], depending on the type of integrin expressed by the myocyte [44, 183]. Both the molecular composition of the ECM [22, 35] and expression levels of different integrin receptors [170] vary during development, suggesting that these two factors co-regulate morphogenesis. For example, fibronectin levels [56, 152] and expression of β1 integrin receptors [34] are elevated in developing myocardium compared to adult, which biases myocytes to spread [89] and proliferate [93] during early stages of morphogenesis. Further functional diversity of integrin receptors is provided by differential splicing of mRNA during development [47]. For example, the β1 splice variant β1A is expressed in embryonic myocardium but β1D is expressed in the adult heart, possibly because β1D provides stronger attachments to the ECM in response to increased myocyte force generation [12, 179]. Together, these observations suggest that complex interactions between the molecular composition of the ECM and the expression patterns of integrin subunits co-regulate cytoskeletal remodeling during cardiac development by structurally stabilizing the cell and regulating proliferation, migration, and signaling with spatiotemporal control.

In summary, these in vivo studies allude that the cytoskeleton plays a prominent role in coupling extrinsic biomechanical forces and biochemical signals from the ECM with intrinsic remodeling of myocyte shape during development, which collectively impacts whole organ morphogenesis. Therefore, cytoskeletal architecture is not passive, but instead actively regulates cardiac morphogenesis and is sensitive to the extracellular environment. However, in some conditions, the cytoskeleton cannot appropriately respond to changing microenvironmental stimuli, which leads to pathogenesis.

Myocyte shape and pathological development

Several cardiomyopathies are characterized by increased mechanical loads that induce both gross morphological changes of the ventricle and dysregulation of ventricular myocyte shape [6, 73–75]. For example, pressure overload causes thickening of the ventricular chamber, known as concentric hypertrophy [105]. This phenotype is attributed to increased cross-sectional area of ventricular myocytes [133, 160, 182, 189] due to the parallel addition of myofibrils [83], which decreases the length to width ratio from 7:1, as seen for normal ventricular myocytes, to approximately 5:1 or smaller [75]. Conversely, in response to volume overload, eccentric hypertrophy develops coincident with the addition of sarcomeres in series, which lengthens ventricular myocytes without changing the cross-sectional area (Fig. 2c, d) [13, 33, 76, 83]. This form of remodeling increases the length to width aspect ratio to 11:1 [75] concurrent with thinning of the ventricular wall, dilation of the chamber (Fig. 2e) [118], and elevated wall stress [13, 76, 83], which only further mechanically loads the myocytes and induces continuous maladaptive remodeling of the cytoskeleton. Both concentric and eccentric hypertrophy are associated with reduced contractility and eventual heart failure [105], suggesting that optimal cytoskeletal contractility is dependent on maintaining the length to width aspect ratio of ventricular myocytes within a narrow window.

In addition to mechanical overload, diseases associated with abnormalities in ventricular myocyte shape and chamber morphology can be rooted in genetic mutations for sarcomeric proteins. For example, dilated cardiomyopathy can be attributed to mutations for genes that encode sarcomeric proteins needed for force generation, such as β-myosin heavy chain, troponin T [2, 104], and troponin I, [132] or force transmission to the ECM, such as dystrophin [111]. These studies imply that genetic mutations which compromise contractility or mechanotransduction prohibit the cytoskeleton from effectively responding to mechanical loads and maintaining cardiac function. This example illustrates how the feedback between mechanical input and intrinsic remodeling can fail when myocytes have defective cytoskeletal elements that inhibit adaptive remodeling.

Maladaptive ECM remodeling is another factor contributing to pathological growth. Similar to development, both the composition of the ECM [134, 153] and expression of different integrin receptors [148] are modulated in disease. Pressure overload has been associated with both re-expression of fibronectin [151] and increased levels of β1-integrin [8, 134]. Similarly, the remodeling phase that follows myocardial infarction is associated with accumulation of fibronectin [177] and differential expression of α1, α3, and α5 integrins [131]. Because integrins regulate many processes within the cell, including cytoskeletal remodeling, their differential expression during disease, combined with alterations in the ECM, may play an important role in maladaptive cardiac remodeling.

In the examples described above, adaptive remodeling of the cytoskeleton fails because mechanosignals are aberrant, genetic mutations limit cytoskeletal adaptation and function, or remodeling of the ECM triggers pathological growth. Each of these implicates maladaptive cytoskeletal remodeling as a contributor to organ malfunction, a notion suggested by gross pathological changes of ventricular morphology concurrent with geometric changes of individual ventricular myocytes. Collectively, these correlations between cytoskeletal remodeling and development and disease have motivated studies to understand cytoskeletal sensitivity to mechanical stimuli.

Cytoskeletal mechanosensitivity

The cytoskeleton responds to mechanical inputs with sensitivity and specificity due to a diverse collection of mechanosensory strategies. As shown in Fig. 3, many mechanosensing complexes directly link specific features of the extracellular environment to distinctly oriented myofibril elements, allowing the cytoskeleton to detect both the source and direction of mechanical loads and initiate appropriate responses within the myocyte. For example, integrins attach Z-discs laterally to the ECM at costameres, cadherins link together terminating myofibrils of neighboring cells at adherens junctions, and titin resides within the sarcomere itself, spanning between Z-discs and M-bands. These three protein structures function as mechanosensors for extracellular, intercellular, and intracellular mechanical stimuli, respectively. Here, we will briefly describe how integrins, cadherins, and titin function in mechanotransduction.

Integrins, costameres, and extracellular mechanics

In most cell types, integrins couple the ECM to the ends of actin filaments via several linker proteins at focal adhesion

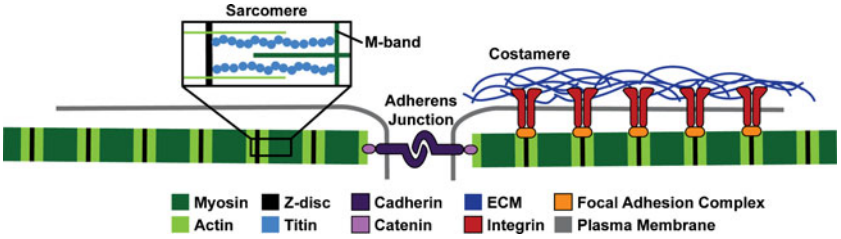

Fig. 3 Mechanosensing in cardiac myocytes. Cardiac myocytes have many mechanosensors associated with the actomyosin cytoskeleton. At costameres, integrins and focal adhesion complexes link the ECM to the Z-disc and detect extracellular stress. Cadherin/catenin complexes link the ends of myofibrils from neighboring cells to detect intercellular loads at adherens junctions. Within the sarcomere, titin spans between the Z-disc and M-band and acts as an intracellular strain sensor. Because these mechanosensors have different ligands and orientations relative to the axis of alignment, the cytoskeleton can discriminate the direction and source of mechanical inputs

plaques [10, 71]. Integrins in the adult heart, however, are primarily localized to costameres (Fig. 3), which are striated muscle-specific transmembrane protein complexes that provide structural stability by anchoring Z-discs laterally to the ECM [4, 45]. Because integrins are mechanotransducers [26, 126], both focal adhesions and costameres are sensitive to mechanical loads from the ECM [94] and interact with many signaling molecules, such as focal adhesion kinase [174] and integrin-linked kinase [14]. Another protein associated with costameric mechanosensing is melusin, a protein that binds directly to the cytoplasmic tail of β1-integrin at costameres [25] and participates in stretch-activated signaling pathways for protein synthesis and hypertrophy [24]. Melusin-null mice suffer from maladaptive eccentric hypertrophy in response to transverse aortic coarctation [24], indicating that melusin is essential for adaptive remodeling in response to increased mechanical load. Muscle LIM protein (MLP) is another costameric protein implicated as a mechanosensor [61]. Although the role of MLP is not completely understood, it is thought to stabilize the T-cap/titin interaction at the Z-disc to allow titin to sense and respond to mechanical stresses transmitted from the ECM [20, 108]. Consequently, mutations in MLP are associated with dilated cardiomyopathy [108] because myocytes are not able to properly sense and adapt to mechanical loads. Collectively, these studies indicate that integrins and costameres play an important role in adaptive remodeling of the heart.

Titin and intracellular mechanics

Titin is an extensive, highly elastic protein [86] that spans Z-discs and M-bands within sarcomeres (Fig. 3) [66] and is closely associated with both thick and thin filaments [49]. Due to its elasticity, titin functions as a molecular spring that supports sarcomere recoiling after systole [87]. In addition to its mechanical role, titin also regulates gene expression by interacting with a wide variety of molecules involved in many different signaling pathways [109, 116]. Because titin is mechanically deformable, active in signaling, and directly linked to sarcomeric elements, it is uniquely equipped to serve as a mechanosensor sensitive to sarcomere length and intracellular strains with control over mechanotransduction.

Several mechanisms have been proposed to explain how titin functions as a mechanosensor [115, 116]. For example, muscle ankyrin repeat proteins (MARPs) are activated for signaling and nuclear regulation of transcription after binding between specific domains of the titin molecule in the elastic I-band region [127]. Titin–MARP binding is enhanced when myocytes are stretched, suggesting that intracellular strain extends titin and allows the MARP binding site to become more accessible [127]. MARP expression levels increase with both pressure overload hypertrophy [3] and dilated cardiomyopathy [130], indicating that MARPs are indeed sensitive to increased mechanical load. Additionally, four-and-a-half-LIM-domain (FHL) proteins, which activate transcription and signaling [116], also bind to the elastic I-band region of titin and are thought to play a role in mechanosensing [155, 110]. Studies have shown that deleting specific I-band domains causes increased strain and loss of elasticity within titin, which is associated with upregulation of FHL protein levels and hypertrophy [82]. These examples demonstrate how the elasticity of titin allows it to function as an intracellular stress sensor by exposing or hiding different binding domains for signaling molecules based on the level of tension applied to the molecule.

Cadherins, adherens junctions, and intercellular mechanics

Cadherins are transmembrane receptors that bind together adjacent cells and link intracellularly to actin filaments via catenins (Fig. 3) [85], providing cadherins with an avenue to transmit mechanical forces from neighboring cells directly to the actomyosin cytoskeleton. Because actin filaments pull against adherens junctions [50, 69, 117], cadherins facilitate the bi-directional transmission of cytoskeletal tension between cells. Studies have demonstrated that cadherins

function as mechanosensors by actively reinforcing cell–cell junctions in response to tugging forces [113, 117], but the mechanism of mechanosensing remains unknown. Collectively, these studies indicate that cadherins mediate the transduction of cytoskeletal tension between cells and respond to intercellular mechanical load by actively remodeling the cytoskeleton to strengthen the adherens junction.

Adherens junctions in the adult ventricle are highly localized to intercalated discs at the longitudinal borders of myocytes, where they bind together ends of myofibrils [5]. Therefore, adherens junctions in the adult sense mechanical loads transmitted primarily from the longitudinal direction, endowing them with directional specificity. In early stages of development, cadherin is also detected in costameric *Z*-disc structures [77, 184, 185], suggesting that cadherins form lateral cell–cell junctions in fetal and neonatal myocardium and therefore also detect mechanical stresses along the transverse axis. Inhibiting cadherin function disrupts myofibrillogenesis [77, 88, 99, 121, 145, 161], suggesting that mechanotransduction at cadherins is essential for synchronizing cardiac development.

Microtubules as mechanosensors?

More recent work suggests that microtubules may play a role in sensing both exogenous and intrinsic forces. The tensegrity hypothesis of cellular architecture suggests that microtubules bear compressive loads, where compressive elements buckle under mechanical loading that exceeds a threshold value [100]. In the heart, a long history of reports suggests that the microtubule network within the myocyte undergoes topological changes during development and disease. For example, pressure overload hypertrophy is associated with microtubule hyperpolymerization and a shift in the balance of depolymerized versus polymerized tubulin [175], which increases the viscosity of the cytoplasm [168] and impedes sarcomere motion [176]. This observation led to the hypothesis that microtubules remodel in response to extracellular stimuli, and the resulting alterations in the network result in increased impedance to systolic shortening of the myocyte [168, 175, 176]. In vitro studies demonstrated that microtubules in beating cardiac myocytes buckle during systole and unbuckle during diastole with a short buckling wavelength [27]. This is important because the shorter buckling wavelength indicates that the microtubules are mechanically coupled to the rest of the cytoskeleton, which structurally reinforces the microtubules to prevent a longer wavelength buckling. Buckling along the length of a microtubule that stretches from the microtubule organizing center to the internal periphery of the myocyte suggests that compressive loads borne by the microtubule are propagated throughout the cytoskeletal network and may contribute to the integration of microcompartmentalized, intracellular responses to mechanical loading.

In summary, the cytoskeleton is embedded with stress sensors that bind unique ligands and associate with specific myofibril elements, which allows the cytoskeleton to detect mechanical stimuli from different sources with directional specificity. Importantly, the structure of the cytoskeleton is sensitive to mechanical inputs, indicating that it first senses microenvironmental cues and then dynamically remodels itself to accommodate the mechanical load.

Microenvironmental cues regulate cytoskeletal architecture: lessons from in vitro studies

Cytoskeletal architecture is sensitive to many insoluble factors in the extracellular microenvironment, such as cell shape, components of the ECM, neighboring cells, stiffness, and topology. During normal growth, these cues are highly regulated in both space and time to cooperatively guide the proper development of cardiac structure and function. When these cues are thrown out of balance by pathological stimuli, they can trigger maladaptive reorganization of the cytoskeleton and disease. Because the microenvironment in the heart is heterogeneous, investigators have exploited in vitro models to control parameters during experiments designed to recapitulate cardiac myocyte structure and function. Here, we summarize the effects of different elements of the microenvironment on cytoskeletal architecture.

Cell shape

New technologies in cellular engineering enable investigators to control cell shape in vitro to understand how cell geometry dictates intracellular structure [55]. One method, microcontact printing, entails patterning ECM proteins onto culture substrates in user-defined patterns of cell adhesion [38, 129]. Using this method, we have learned that myocyte shape is very important in regulating the placement and overall alignment of the contractile actomyosin cytoskeleton [28, 72]. The role of extracellular boundary conditions in regulating myofibrillogenesis has been studied to understand how variations in myocyte shape, observed with different cardiomyopathies, are accompanied by alterations in sarcomere alignment (Fig. 4) [28]. These studies register with both clinical studies in humans and experimental models of cardiomyopathies that report alterations in ventricular myocyte shape concurrent with sarcomere disarray and contractile failure [75].

Cell shape regulates the self-organization of cytoskeletal architecture and coupling to the extracellular matrix. In studies of several cell types cultured on islands of extracellular matrix, focal adhesions assemble at cell borders, often at locations of

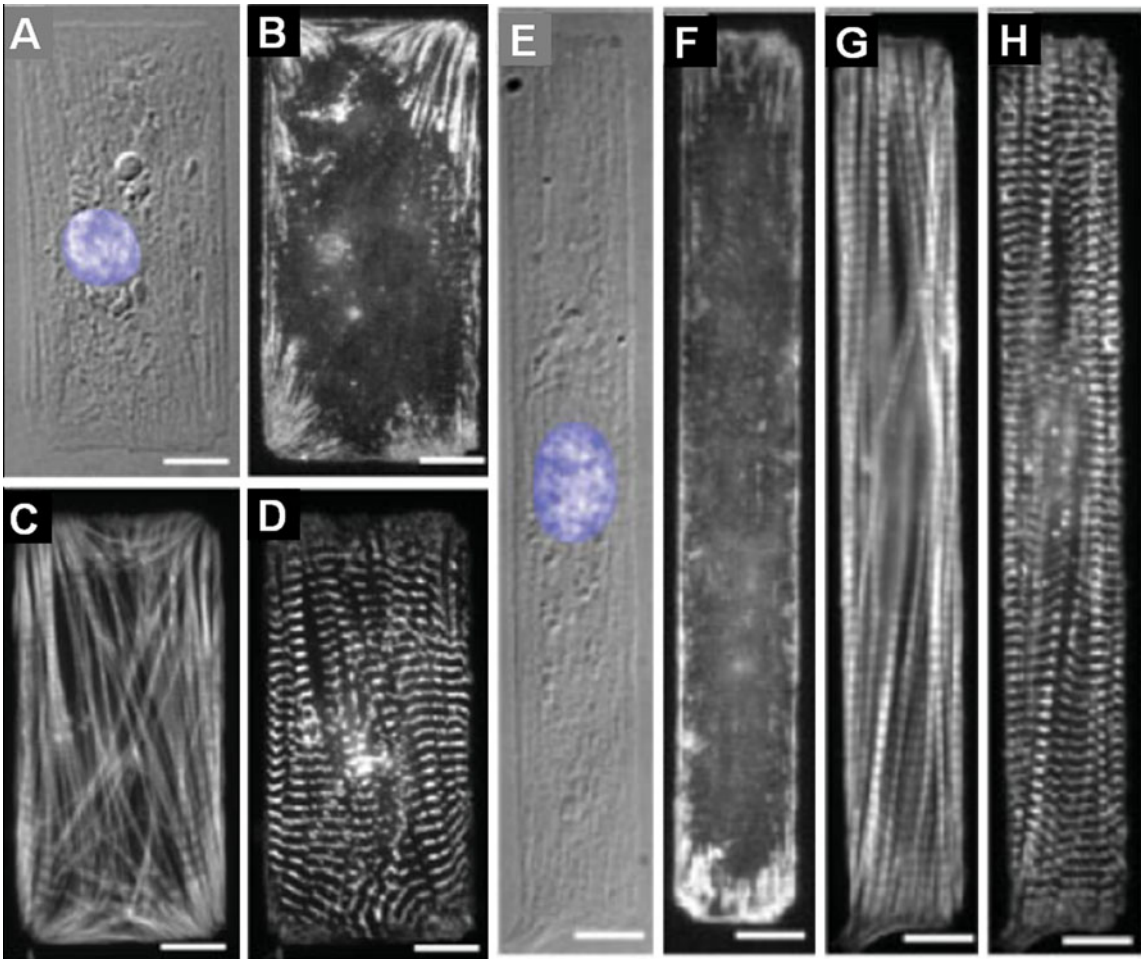

Fig. 4 Cell shape dictates cytoskeletal architecture. Micropatterned myocytes with length to width ratios of 2:1 (**a**–**d**) and 7:1 (**e**–**h**) self-assemble their cytoskeleton based on the boundaries of the ECM pattern. Focal adhesions extend in a more radial pattern in 2:1 myocytes (**b**, vinculin) compared to 7:1 myocytes (**f**, vinculin). Consequently, myofibrils (**c**, **g**, actin; **d**, **h**, α-actinin) are better aligned with the long axis in 7:1 myocytes. This has implications for predicting how remodeling of myocyte shape affects cytoskeletal architecture and contractile function during physiological and pathological growth. Modified from [28]

abrupt change in boundary curvature [36]. Similarly, patterned rectangular myocytes with length to width aspect ratios ranging from 2:1 to 7:1 attach to the substrate via focal adhesions almost exclusively at the longitudinal borders of the cell (Fig. 4) [28]. Together, these results suggest that the spatial organization of focal adhesions and attachment to the extracellular matrix is tightly controlled and sensitive to extracellular boundary conditions.

ECM composition

Culturing myocytes at different developmental stages on substrates coated with ECM [119] has allowed investigators to understand how myocyte–ECM interactions regulate cardiac structure and function during physiological and pathological growth. For example, neonatal myocytes attach to substrates coated with laminin, fibronectin, or collagen types I–V, but previous reports suggest that adult myocytes attach only to substrates coated with laminin or type IV collagen and attach weakly to fibronectin [23, 120]. These studies imply that neonatal and adult myocytes have different integrin receptors, suggesting that integrin expression is developmentally regulated, a reasonable assumption given the well-documented changes in the extracellular matrix during development and disease. Furthermore, myofibril assembly [90, 91, 159] and signaling [29] are regulated by the composition and orientation of the ECM, indicating that developmental signals are embedded within the ECM and suggesting that the ECM represents a signaling network by which cells can communicate in a manner analogous to the diffusion of soluble mitogens in paracrine signaling networks. In one illustrative example, the functional role of β1 integrin in development and disease has been studied by blocking its function in cultured myocytes, revealing that β1 integrins are essential for differentiation [57], spreading and myofibrillogenesis

[89], proliferation [93], and hypertrophy [142, 149]. These studies suggest that ECM regulation over myocyte structure and function is developmentally co-regulated by both the expression of different integrin receptors on the myocyte surface and the molecular composition of the ECM.

Cell–cell contacts

Cell–cell adhesion triggers global reorganization of cytoskeletal structure mediated by interactions between actin filaments and the cadherin–catenin complex at the adherens junctions [85]. The dynamics of this process have been extensively studied in cell culture systems, where the use of green fluorescent protein, knockout models, and time-lapse microscopy have been valuable tools for gaining insight into how adherens junctions regulate cell and tissue morphogenesis. In cultured epithelial cells, membrane contact initiates recruitment of cadherin receptors [1], forming adherens junctions that actively expand to maximize cell–cell contact [186]. Myocytes in culture follow a similar process of adherens junction formation and expansion, inducing global reorganization of cytoskeletal structure and myocyte form as cell–cell contact is maximized [190]. Adherens junctions are crucial for coordinating myofibrillogenesis across developing tissues, as inhibiting N-cadherin function severely disrupts the assembly and maintenance of myofibrils [77, 88, 99, 121, 145, 161]. Additionally, the amount of cell–cell contact positively correlates to survival time and maintenance of spontaneous contractility in cultured myocytes [42], demonstrating that adherens junctions are vital for developing and maintaining a functional cardiac phenotype. Myocytes can also establish functional linkages with non-myocyte cell populations [70, 139], indicating that interactions with other cell types may also mediate cardiac tissue morphogenesis. Together, these studies indicate that adherens junction formation is an active process that mediates global cytoskeletal architecture and is essential for coordinating myofibrillogenesis and the formation of a cohesive, functional tissue.

In adult myocytes in vivo, adherens junctions localize to longitudinal borders in intercalated discs and are absent from lateral borders [79], as opposed to fetal and neonatal myocytes, which have cadherins distributed around all borders [5, 80, 141]. Polarization of adherens junctions coincides temporally with birth when cardiac output increases dramatically to support the needs of the newborn organism [81] and myocytes elongate and hypertrophy [92]. Therefore, the polarization of adherens junctions is coincident with increased force generation, suggesting that maturation of intrinsic contractility contributes to the isolation of cadherins to longitudinal borders. This hypothesis is supported by in vitro studies, which suggest that adherens junctions are positively responsive to biomechanical forces [113, 117, 150, 157, 188]. Conversely, lateral borders would be subjected to high shear forces, which could potentially prevent the formation and maintenance of adherens junctions. Therefore, because adherens junctions are responsive to tension, the polarization of adherens junctions to intercalated discs at the longitudinal borders could be induced by the maturation of intrinsic contractility following birth, suggesting that cytoskeletal function feeds back to adherens junction form.

Stiffness

Extracellular rigidity has also been thought to contribute to cytoskeletal remodeling. In vivo, cardiac tissue becomes mechanically less compliant during development [173] and some cardiomyopathies [16]. Correlations between substrate stiffness and cytoskeletal structure have been explored in vitro with many cell types, where integrin receptors have been shown to sense mechanical properties of the ECM and induce cytoskeletal remodeling to match intracellular tension to the extracellular load [101, 114]. For example, fibroblasts strengthen their cytoskeletal linkages to optically immobilized fibronectin-coated microbeads specifically bound to integrins in proportion to the amount of resistance applied to the bead [39]. This type of remodeling consists of an immediate viscoelastic response followed by distinct stages of cytoskeletal remodeling on timescales ranging from seconds to minutes [125], indicating that the cytoskeleton is highly adaptive to mechanical loads applied at focal adhesions.

Cardiac myocytes similarly remodel their actomyosin cytoskeleton in response to stiffness, which has been studied in vitro by culturing myocytes on substrates with tunable elastic moduli [140]. On soft substrates with an elastic modulus of 1 kPa, myofibrils generate low levels of tension because the substrate provides little resistance to focal adhesion attachments, thereby limiting myocyte spreading, cytoskeletal strengthening, and myofibril formation [9, 17, 52, 103]. Excessive rigidity also appears unfavorable for myofibril assembly, as indicated by decreases in traction forces, beating rate, and appearance of striated myofibrils in myocytes cultured on substrates with an elastic modulus of approximately 50 kPa [9, 17, 52, 103]. Substrates with moderate elastic moduli of 10 kPa appear ideal for myofibril maturation and force generation [9, 17, 52, 103], somewhat similar to the 18 kPa elastic modulus of myocardium in vivo [16], suggesting that physiological stiffness is important for enhanced myofibril formation and contractility. Infarcted myocardium has an elastic modulus of approximately 55 kPa due to increased fibrosis [16], which may contribute to diminished contractile function. Other processes within the cell, such as calcium handling, are also dependent on substrate stiffness [103], illustrating how rigidity can regulate the expression and function of non-cytoskeletal proteins as well. Linking tissue stiffness measurements during develop-

ment and disease to in vitro observations of myofibrillogenesis on different elastic moduli implies that the cytoskeleton detects rigidity and remodels itself appropriately to endure the extracellular load.

Topology

In vitro studies suggest that cardiac tissue form is also regulated by variations in topography. For example, neonatal rat ventricular myocytes align into anisotropic tissues on surfaces engraved with either nanoscale [106] or microscale [18, 54] grooves. This has many implications for cardiac morphogenesis in vivo because the extracellular environment in the ventricle is full of structural heterogeneities, such as blood vessels, nerves, fibrotic tissue, etc., each of which provides a topographical cue that could influence cell and tissue structure.

In summary, in vitro studies have allowed investigators to understand how individual aspects of the microenvironment directly influence cytoskeletal remodeling, indicating a feedback loop between mechanosensing and cytoskeletal adaptation. The cytoskeleton also transmits mechanical forces to regulate other processes within the cell, indicating that cytoskeletal remodeling is responsible for linking the extracellular microenvironment to a wide range of cellular functions.

Cytoskeletal architecture regulates function

As described above, the cytoskeleton is highly sensitive and adaptive to mechanical forces and structural cues. The cytoskeleton also regulates many other processes within the cell, including signaling, excitability, impulse propagation, contractility, and gene expression. Therefore, the cytoskeleton acts as a signal integrator for mechanical and structural inputs that then transmits these information throughout the myocyte to ensure that other processes within the cell respond and adapt appropriately. In vitro studies have again been useful for understanding how cytoskeletal architecture mediates diverse cellular functions.

Signaling

By manipulating the surface chemistry of cell culture substrates [55], investigators can regulate cell shape in vitro and quantify functional responses. In one study, the adhesivity of tissue culture plastic was modulated by coating the surface with serial dilutions of a polymer that hampered cell spreading [62]. Quantifying DNA synthesis with ^{3}H-thymidine incorporation revealed that endothelial cell growth was proportional to cell size [62], demonstrating that cell shape directly regulates proliferation. With micropatterning, Ingber and colleagues engineered the shape of capillary endothelial cells to show that cellular form regulated genetic programs governing cell growth, differentiation, and apoptosis [37]. This study also showed that cell survival is independent of the quantity of cell–ECM attachments, eliminating focal adhesion formation and integrin signaling as the main cause of enhanced cell survival and further implicating cell shape as a critical regulator of cell growth [37].

Because cells self-assemble their cytoskeleton with respect to extracellular boundary conditions [36, 137], cell shape spatially organizes the placement of protein complexes that tend to be restricted within cellular microcompartments. For example, focal adhesions consistently localize to the corners of square cells [135]. These large protein complexes transmit tension between the cytoskeleton and the ECM [10] and are hubs of cell signaling [154]. Rac activation and lamellipodia extension are also highest at corners [135], indicating subcellular spatial segregation of signaling and motility based on ECM boundaries, cell geometry, cytoskeletal architecture, and distribution of mechanical forces. Collectively, these studies show that cell shape is a functional cue that actively regulates growth, apoptosis, signaling, and motility.

Excitability

Extrinsic mechanical forces regulate electrophysiology through mechanisms of mechano-electrical coupling thought to be mediated by the cytoskeleton. For example, pressure or volume overload stretches the ventricle, which is associated with shortening of action potential duration [64], slight depolarization of resting membrane potential [21], premature ventricular excitations, and stretch-activated arrhythmias [64, 65]. These observations have prompted studies into the hypothesis that the cytoskeleton transmits mechanical forces to ion channels to regulate their behavior. One such study demonstrated that microtubule depolymerizing agents increase the probability that L-type Ca^{2+} channels are in a closed state, whereas microtubule-stabilizing agents increase the probability that they are in an open state [68]. Consequently, hearts treated with microtubule stabilizing agents are more susceptible to stretch-activated arrhythmias [138]. The actin cytoskeleton has also been linked to ion channel behavior, as actin filament disruptors affect the gating of K(ATP) channels [67, 171]. Collectively, these studies suggest that cytoskeletal transmission of mechanical forces modulates ion channel activity and excitability.

Perturbation of the cytoskeleton alters ion channel kinetics, suggesting that different cytoskeletal architectures provide myocytes with unique electrophysiological properties. To study this, investigators have used in vitro models to compare measurements of ion channel behavior and

calcium handling in cultured myocytes with different tissue architectures. The expression of the calcium channel α1C subunit and peak values for I_{to}, I_{Na}, and I_{Ca} are all increased for aligned neonatal rat ventricular myocytes compared to pleomorphic cultures [181]. Furthermore, tissues aligned with microgrooved substrates show increased systolic intracellular Ca^{2+} and decreased diastolic rise time [187]. These studies suggest that cytoskeletal architecture can regulate electrophysiology and furthermore indicate that an aligned phenotype similar to that observed in vivo is optimal for ion channel expression and function.

Impulse propagation

In addition to regulating excitability, both cell shape and tissue organization also mediate impulse propagation. Although conduction is essentially continuous through bulk cardiac tissue on the macroscopic scale [107], optical mapping of myocyte strands stained with voltage-sensitive dyes has revealed a significant delay in propagation across cell borders on the microscopic scale [58] mediated by low resistance gap junction channels composed primarily of connexin 43 [11, 172]. Because cytoplasmic resistance is significantly lower than gap junction resistance [58], cell shape and tissue organization are powerful regulators of impulse propagation by dictating the amount of resistance to current flow in different directions based on the relative number of cell borders. In silico work has suggested that the size of myocytes regulates propagation to the same or even greater extent as gap junction localization due to the large differences in cytoplasmic and gap junction resistances [163, 164]. Furthermore, in vitro optical mapping studies of aligned monolayers of neonatal myocytes have demonstrated that the anisotropy ratio of propagation is heavily dependent on tissue organization and alignment [31, 40, 106], even though gap junctions are not localized to the longitudinal borders in these cultures [5, 80]. Taken together, these studies indicate that, in addition to gap junction distribution, rapid electrical propagation in ventricular tissue is dependent on the size, shape, and orientation of myocytes due to substantial differences in the resistance to current flow within and across cell borders. How cytoskeletal architecture and mechanical coupling of these cells affect action potential propagation has yet to be determined, but may offer a means of acute regulation of cell–cell electrical coupling.

Contractility

Tissue organization affects the contractile performance of engineered tissues in vitro by aligning myofibrils and their sarcomere units uniaxially. For example, anisotropic monolayers of neonatal rat ventricular myocytes aligned with either nanotopography [106] or micropatterning [59] contract unidirectionally, thereby generating more force than pleomorphic cultures that generate force in random directions. In addition to improved sarcomere alignment, tissues aligned with structural cues also develop a hypertrophic phenotype, characterized by increases in cell size and force generation [41]. Therefore, elongation of myocytes affects contractility by two mechanisms: (1) uniaxial alignment of sarcomeres and (2) genetic reprogramming to a hypertrophic phenotype.

Gene expression

In response to cyclic mechanical strain, cardiac myocytes in vitro become hypertrophic, as signified by increases in cell size and overall protein expression [19, 32, 46, 158] and the activation of fetal gene programs [63]. Therefore, cyclic stretch is an extrinsic cue that induces intrinsic changes in gene expression, presumably to equip the myocyte to better cope with the mechanical load. However, certain stretch-mediated processes are dictated by the direction of stretch relative to the axis of tissue alignment. For example, protein turnover, myofibril injury [158], expression of hypertrophy markers, Cx43, and N-cadherin [78], and protein kinase C activity [30] are all upregulated when stretch is applied transversely compared to longitudinally. The mechanism for directional dependency could be due to the specific placement and orientation of mechanosensors relative to cytoskeletal alignment. For example, integrins are localized to costameres, which are perpendicular to myofibrils, but titin is found inside the sarcomere, parallel to myofibrils. The differential activation of these mechanosensors could endow the cytoskeleton with the ability to detect the direction of mechanical loads and activate different programs of gene expression, providing myocytes with better adaptability.

Because cytoskeletal architecture regulates many aspects of myocyte function, investigators have attempted to steer the gene expression of undifferentiated cells by exposing them to the same microenvironmental cues as differentiated myocytes. For example, the alignment of mesenchymal stem cells [143, 169] or progenitor cells [51] drives them towards a myocardial lineage, suggesting that an elongated morphology is an intrinsic cue for differentiation. Extrinsic mechanical inputs also regulate differentiation, as cyclically stretched embryonic stem cell-derived cardiomyocytes increase their expression of cardiac-specific genes [84, 156] and myogenic differentiation of mesenchymal stem cells is highly sensitive to substrate stiffness [53]. These studies suggest that gene expression programs for differentiating stem cells and progenitor cells are guided by microenvironmental cues that are propagated through the cytoskeleton.

Conclusions

In summary, microenvironmental cues, including cell shape, the ECM, neighboring cells, substrate stiffness, and topology, are detected by specialized protein structures embedded within the cytoskeleton, such as costameres, adherens junctions, and titin. After sensing mechanical inputs, the cytoskeleton remodels its architecture to adapt to the extracellular boundaries and extrinsic mechanical loads. The cytoskeleton also propagates mechanical inputs from the microenvironment to mediate other functions within the myocyte, including signaling, excitability, impulse propagation, contractility, and gene expression.

In vitro studies have been widely used to study how mechanical forces impact myocyte structure and function. While these studies have been informative for understanding how myocytes sense and respond to mechanical inputs, the challenge lies in synthesizing all the information gained from in vitro models and relating it to processes of development and disease in vivo. One of the major limitations of most in vitro models is the use of two-dimensional tissues to study processes that normally occur in three dimensions. For example, the distribution of focal adhesions and adherens junctions is very different in an essentially flat monolayer compared to a three-dimensional mass of myocardium. As tissue engineering continues to advance, it will become easier to study mechanotransduction in three-dimensional models that better recapitulate the environment of native myocardium.

In conclusion, mechanotransduction is especially complex in the heart because of the many types of dynamic mechanical loads applied to myocytes. The cytoskeleton is the primary means of sensing, integrating, and coordinating cellular responses to mechanical stimuli and structural cues. To better understand mechanotransduction, it will be necessary to study it with a systems approach in order to synthesize information learned from studies done on multiple size scales, from the organ down to the single protein level.

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