This Review is part of a thematic series on **Mechanotransduction and Signaling in Myocardium**, which includes the following articles:

Role of Integrins in Endothelial Mechanosensing of Shear Stress

Dance Band on the *Titanic:* Biomechanical Signaling in Cardiac Hypertrophy

Spatial Microstimuli in Endothelial Mechanosignaling

Dynamic Regulation of Connexins in Connection With Mechanosignaling

Peter F. Davies, Guest Editor

Dance Band on the *Titanic*

Biomechanical Signaling in Cardiac Hypertrophy

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Abstract—Biomechanical signaling is a complex interaction of both intracellular and extracellular components. Both passive and active components are involved in the extracellular environment to signal through specific receptors to multiple signaling pathways. This review provides an overview of extracellular matrix, specific receptors, and signaling pathways for biomechanical stimulation in cardiac hypertrophy. **(*Circ Res*. 2002;91:888-898.)**

Key Words: mechanical signaling ■ cardiac hypertrophy ■ extracellular matrix

While all cells, tissues, and organisms depend on external mechanical inputs to maintain their phenotypes, cardiac cells are also constantly generating active forces of contraction. Mechanical forces are intimately linked to chemical signals that lead to many interrelated modifications both inside and outside the various cell types of the heart. Outside the cells, these modifications include dynamic restructuring of the extracellular matrix (ECM) and its signaling components and alterations in the quantity and type of cell surface receptors. Within the cell, changes occur in signaling proteins, contractile apparatus, and cytoskeleton. Because these structural alterations prompt alterations in mechanical properties of the cells and ECM, they result in a redistribution of mechanical stimuli until a new (mechanical and biological) equilibrium is achieved. This dynamic system works well in the physiological adaptation to a variety of signals that result in normal cardiac growth or development; however, when this equilibrium goes out of balance, the result can be pathological growth and abnormal physiology. In this review, we focus on the components that regulate biomechanosignaling in the cardiac myocyte and their integration.

Mechanical stress provides critical information for maintenance of myocardial structure and function. Because dynamic changes in stress occur with every contraction/relaxation cycle as well as with short- and long-term hemodynamic alterations, this information must be integrated using multiple sensors. Chronic elevation of force exerted on cardiomyocytes prompts signaling reactivity and remodeling that transforms the structural characteristics and physical properties of the myocardium altering the balance of forces between different regions and components of the tissue. Increased physical stress on the heart can be normal and beneficial, but pathological conditions such as hypertension, ischemia, or contractile abnormalities that increase stress excessively lead to maladaptive remodeling that may precede the onset of heart failure. Because of the close interplay between the myocytes and the ECM, it is not surprising that cellular and extracellular remodeling tends to occur in tandem, such as fibrosis that accompanies pressure-overload hypertrophy.

Mechanotransduction—the transformation of a mechanical stimulus into cellular responses—is a hallmark of myocardial cells. Mechanical stress and strain activate a panoply of signaling pathways too numerous to be adequately addressed in this review. Accordingly, the overview presented here is

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not intended to be exhaustive or highly detailed. Rather than reiterate the perspective of recent related reviews,[1–3] the purpose herein will be to concentrate on signaling pathways associated with structural remodeling in response to altered mechanical loading of the myocardium and to provide an integrated view of how mechanical stimulation cascades into promotion of cellular growth, remodeling, and survival.

Organization of the ECM

Intimately associated with mechanical force are the components of the ECM and their organization in the interstitium. Initially described as structural molecules with a passive role in force transmission, the ECM is now recognized to play a more dynamic role in signaling cellular growth, apoptosis, and remodeling. The ECM is now viewed as a composite network of large molecular weight collagens, proteoglycans, glycosaminoglycans, and glycoproteins that act both as structural components and in the regulation of cell function.[4] In addition, a subset of ECM components called matricellular proteins has been proposed.[5] These are critical in the regulation of cell growth, migration, apoptosis, and phenotype and include growth factors, cytokines, and proteases. These in turn regulate cell-matrix interactions, playing an important role in mechanical signaling.

The principal structural components of the ECM are the collagens, which form a three-dimensional network interconnecting myocytes to each other and the vasculature. The development of this elastic stress-tolerant collagenous network is intimately associated with the generation and transmission of mechanical forces to and from the myocytes.[6–8] In addition to interstitial collagen, collagen type IV is localized to the basement membrane and is probably important in the regulation of cell volume. Type IV collagen has a chicken-wire arrangement that changes with the contraction of the myocyte and helps maintain cell shape.[9] Thus, the ECM contains both active and latent signaling components that are essential in homeostasis, remodeling in response to pathophysiological signals (growth factors), activation of cell surface receptors (integrins), and remodeling of the collagenous network.

Mechanical Interactions Between Myocytes and the ECM

Cardiac cells are constantly loaded by external forces while simultaneously developing internal contractile forces. The two principal mechanical pathways are the transmission of contractile forces via the sarcomeric lattice, cytoskeleton, and ECM to the ventricular chambers; and the responses of cardiac cells to external hemodynamic loads and to internal stresses developed by the neighboring cells and ECM. These internal and external forces must balance each other to maintain the myocardium in physical equilibrium.

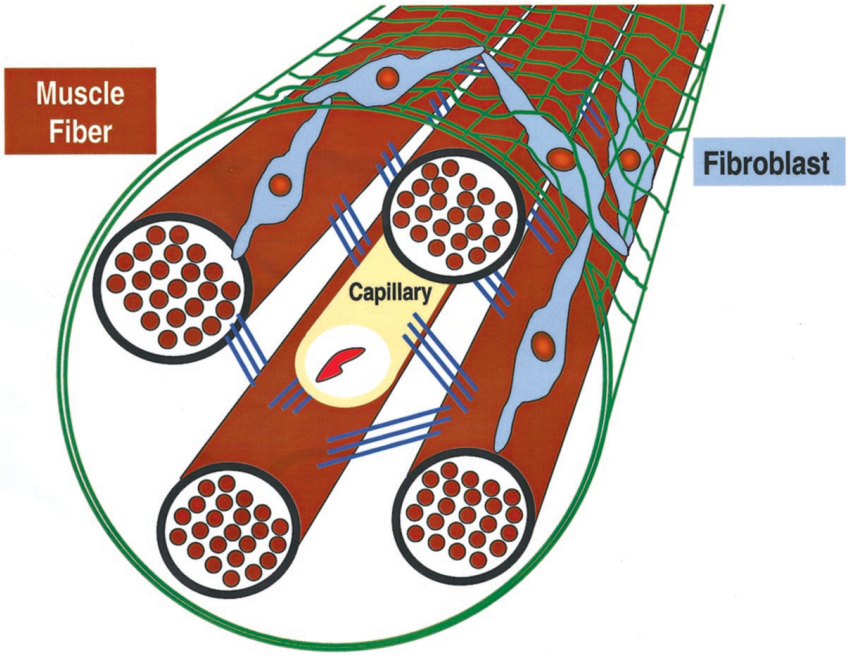
Because the cells and ECM of the myocardium are organized in a three-dimensional hierarchy that supports a nonhomogeneous distribution of stresses, different components of the ECM hierarchy may be structurally in parallel or in series with the cellular components of the myocardium. For example, diastolic fiber stresses are partitioned between myocytes and the collagen network acting structurally in parallel so that they share the total load. The combined passive fiber stiffness of myocardium has been attributed predominantly to intracellular structures, notably titin, at low sarcomere lengths.[10] This giant protein or its binding partners in the cytoskeleton and sarcomere may therefore act as intracellular sensors of fiber stress or strain.[11]

At longer sarcomere lengths, parallel collagen fibers bear an increasing fraction of the axial stress as coiled perimysial fibers untwist and straighten.[12] It is unlikely that these large fibers ever become completely straight even at pathophysiologically high filling pressures.[13] Conversely, during systole, active fiber shortening below the stress-free sarcomere length is opposed by the viscoelasticity of the collagens and proteoglycans.[14] While compression of parallel coiled perimysial fibers stores elastic energy that is released during early filling, there is also a contribution from forces distributed in series between the cells and matrix.[15,16] Elastic energy is developed in transverse collagen "ties" that are strained by the large thickening and transverse shearing deformations of the ventricular walls as myocytes shorten and myofiber lamellae reorient during systole.[17,18] (Note that the endomysial ties are often called "struts," a term that is more correctly reserved for compression-bearing members of a structure.)

Although transverse systolic strains can be large, the usual assumption has been that myofilaments develop significant contractile stresses predominantly or exclusively along their principal axis in the direction of the myofiber axis. However, even simple considerations of crossbridge geometry suggest that up to 30% of the crossbridge force could be directed perpendicular to the fiber axis.[19] How these forces integrate to resultant cellular stresses depends primarily on the sarcomere lattice structure. Tonically activated isolated rabbit septum stretched equibiaxially generates active stresses in the transverse direction that can exceed 40% of those in the fiber direction.[20] Allowing for this property in a biomechanical model of whole ventricular mechanics reconciles significant disparities with experimentally observed wall strains in models that assume myocyte tension generation is uniaxial.[21,22] While recent studies suggest that changes in transverse myofilament lattice spacing are unlikely to be a key mechanism of length-dependent activation,[19,23] they are likely to influence transverse stress development.

Stresses transverse to the myofibers are transmitted from myocyte to myocyte at the level of the z-line via endomysial collagen ties. In view of the concentration of signaling complexes clustered at the costamere, where the z-disk meets membrane-associated ECM receptors, this site is a prime candidate for mechanical sensing. Wang and colleagues[24] suggested that the integrin focal-adhesion cytoskeleton complex is a mechanically sensitive signaling organelle, and substantial evidence now supports this conjecture. Indeed, in vitro studies of stretch responses in micropatterned neonatal cardiac myocytes engineered to grow on deformable surfaces in elongated parallel-fibered morphologies have shown that myocyte hypertrophic responses to stretch may be significantly more sensitive to loads applied perpendicular than parallel to the myofilament axis.[25,26]

Physical interactions between the ECM and the myocytes contribute to the development of the residual stresses that



Diagrammatic organization of the collagenous connective tissue of cardiac muscle. Fibroblasts are shown in and around the endomysial collagen network. Collagen is shown with both direct connections to myocytes and capillaries (blue) as the endomysium. A variety of other ECM components would be found surrounding the cells in the interstitial space. Mechanical signaling to the myocytes from the outside-in could come from contraction of the endomysial collagen by the fibroblasts as well as by fluid within the interstitial space.

remain in the myocardium when external loads are removed.[27,28] Compressive residual stresses shorten endocardial sarcomeres in the unloaded ventricle and are balanced by tensile residual stresses that prestretch epicardial fibers.[29] This provides a mechanism for maintaining transmural uniformity of stress and sarcomere length at end-diastole,[30] and changes in residual stress resulting from nonuniform hypertrophy have been described as an adaptive response to hemodynamic overload in arteries. Yet the most striking alterations in myocardial residual stresses have been developmental responses to ECM gene mutations in the tightskin mouse, *Tsk*, and the osteogenesis imperfecta murine, *oim*.[31,32] The increase in myocardial collagen content in the *Tsk* myocardium was associated with an almost complete loss of residual strain, whereas the disruption of type I collagen assembly in the myocardium of mice homozygous for the *oim* mutation was associated with a 3-fold increase. These observations suggest that nonuniform regional hypertrophy in intact myocardium is mediated via ECM-dependent pathways, either directly via cell-matrix signals, or indirectly via their effects on local stresses, or both.

Force Generation in the ECM

The arrangement of collagen ECM into the three-dimensional network surrounding and interconnecting myocytes forms a laminar myocardial organization[33] with a well-described formation and function.[17,34] However, little notice has been given to the interstitial space between the lamellae. It normally contains fluid moving from the capillaries and taken up by the lymphatic system. Lymphatic fluid includes several ECM components such as proteoglycans and glycosaminoglycans that affect interstitial pressure and viscosity. The electro-osmotic properties of the highly charged proteoglycans and glycosaminoglycans affect the mechanical properties of tissue because their sugar components have a high affinity for water, thereby increasing interstitial hydrostatic pressures and altering stress distributions between cells and matrix; however, their precise regulation and functional roles are poorly understood.[35,36] Interstitial fluid movement is governed by several factors including collagen density. It is likely that as collagen density increases, resistance to interstitial fluid movement increases, increasing pressure gradients and altering the shear stresses applied to myocardial cells and the balance of forces within the myocardium (Figure).

The nonmyocyte cellular components combined with the ECM can also generate forces that mechanically strain the matrix. Experimental models to test the hypothesis of force generation from the ECM primarily use fibroblasts seeded in collagen gels,[37–39] which are allowed to “contract” over time. These studies have demonstrated that gel contraction can be stimulated by a wide variety of factors including growth factors (angiotensin II, insulin-like growth factor-1 [IGF-1], platelet-derived growth factor [PDGF]) and mast cells.[40–42] Contraction can also be blocked by antibodies against integrins, matrix metalloproteases, and pharmacological interventions. While all of these factors are present in the heart during development and disease and could play a role in the exertion of mechanical force to cardiac myocytes, data directly demonstrating their role in vivo are lacking. They could be particularly important in postinfarction wound healing and subsequent chamber remodeling, by contributing to the balance of forces and signals that govern the tendency for scar contraction versus expansion.[43]

The arrangement of fibroblasts in the endomysial collagen is essential to the generation of mechanical force.[44] Studies indicate that fibroblasts exist as a sheet of interconnected cells both in vivo and in vitro in collagen gels. In this arrangement, fibroblasts can transmit electrical or ionic signals via gap junctions and mechanical force by their receptor-mediated connections to the ECM. Interesting experiments have shown that if these connections are blocked, no mechanical force is generated.[16] It would appear that if the fibroblasts

are arranged as a sheet, they are more likely to resist as well as transmit mechanical force to the surrounding myocytes (Figure).

The role of proteases in the generation or redistribution of mechanical stresses in the ECM is relatively unexplored but of significant potential.[45,46] From gel contraction experiments, matrix metalloproteases (MMPs) appear to be an essential component but with an undefined role. ADAMs (a disintegrin and metalloproteases) may also be essential in modification of the ECM in force generation. Other proteases may also contribute by the activation of latent factors such as growth factors or ECM components that would signal growth and thereby generate increased mechanical tension. Although several ECM components, such as fibronectin, have been shown to stimulate myocyte growth (hypertrophy), it is not clear whether this stimulation is as large molecular weight molecules or smaller molecular weight species that have been activated by proteolytic cleavage. Fibronectin may also act as a strain sensor itself by exposing cryptic binding sites as it unfolds under external forces.[47]

Receptors and the ECM

At least three classes of receptors are important in the inside-out and outside-in transmission of mechanical forces between the cells and ECM. They are found on both myocytes and fibroblasts. Integrins are the most well-recognized class of ECM receptor and have been recently reviewed.[48,49] Integrins, which are heterodimers of α and β subunits, change with development, hypertrophy, and aging. The expression of integrins appears to be coordinated with specific ECM expression; however, in heart failure abnormal expression results, which contributes to altered mechanical properties.[50–52] Integrin receptors are involved in signaling in both outside-in and inside-out manner on myocytes and fibroblasts.

A second class of receptors, discoidin domain receptor (DDR), is not well documented but could be significant, because they are transmembrane complexes, attaching specifically to collagen, and have tyrosine kinase motifs in the cytoplasmic domain.[53] There are several isoforms of the DDR family that appear on both fibroblasts and myocytes. However, only DDR2 is found on fibroblasts. This appears to be a unique marker for these cells. A third class of receptors is the cadherins, which regulate cell-cell interaction.[54] Although cadherins play a significant role in myocytes, their role in fibroblasts is not known. However, as fibroblasts within the collagen network appear to be interconnected, this class of receptor could play a significant role in force transmission between fibroblasts.

The arrangement and localization of receptors on the surface of fibroblasts and myocytes appear to be critical to force generation and transmission. In vitro studies show the localization of integrins to be primarily at focal adhesions. This connects the ECM components with the actin cytoskeleton and components of the signaling pathways.[17] Structures analogous to focal adhesions are located in vivo near the sarcomeric Z band and connected to the ECM, cytoskeleton, and signaling complexes. Different from in vitro localization is the presence of the intercalated disk, which may also be important in force transmission and determining the partitioning of stresses between the cells and ECM.

In addition to acting as potentially important slow force generators that regulate the geometry and stress distributions of the myocardium, cardiac fibroblasts, which make up about two thirds of cardiac cells, also have the ability to sense, integrate and functionally respond to mechanical stimuli.[55] They are also a source of autocrine/paracrine factors such as endothelin-1, tumor necrosis factor-α (TNF-α), and angiotensin II that are released in response to mechanical stimulation.[56,57] In vitro studies have shown that induction of ECM gene expression in cardiac fibroblasts by stretch is differentially responsive to dynamic versus static, tensile versus compressive, and uniaxial versus biaxial stretching.[58–60] Upstream, the initial activation of extracellular signal-regulated kinase (ERK) and c-Jun NH_2-terminal kinase (JNK) by stretch is substrate-dependent and integrin-specific.[61]

Autocrine/Paracrine Connections to MAPKs and JAK/STATs

Diverse signaling molecules are activated within minutes of acute mechanical stimulation. Multiple studies have also left no doubt that release of autocrine and paracrine factors amplifies the initial growth stimulus triggered by mechanical stretch. Angiotensin II, endothelin-1, vascular endothelial growth factor (VEGF), and transforming growth factor-β have all been implicated in cardiac cellular hypertrophy in vitro[62,63] and in vivo.[63–67] These growth factors share the common property of directly or indirectly activating members of the mitogen-activated protein kinase (MAPK) family through Raf/Ras-dependent signaling pathways (see reviews[1,68]). MAPK regulation is an established archetype of complex amplification and crosstalk from years of cumulative studies.[69] Briefly, MAPKs are serine/threonine kinases that target substrates in a multilayered signaling network composed of the MAPKs, the MAPK/ERK kinases (MEKs), and the MEK kinases (MEKKs). Among the MAPK superfamily, the ERKs, JNKs, and p38 kinases are well-known mediators of hypertrophic signaling in myocardial cells. MAPKs phosphorylate target substrates in the nucleus (eg, c-myc, c-jun, and ATF-2), leading to transcriptional reprogramming that is likely responsible, at least in part, for altered gene expression associated with hypertrophy.

MAPKs respond to a variety of activating stimuli originating from a diverse array of signaling pathways. For example, ERK can be activated by Raf via Ras, phospholipase C and protein kinase C, protein kinase A, and G protein–coupled receptors stimulated by bound receptors for hormones or growth factors.[68,69] A different pathway involving integrin-FAK-Src-Ras signaling associated with mechanical stretch leads to p38 activation.[70] Thus, although the necessity of the angiotensin II pathway for ERK and p38 activation after stretch has been questioned,[71,72] plenty of alternative ways exist for these MAPKs to become involved in the response to mechanical stretch. For ERK, this includes the transmembrane glycoprotein gp130, which not only activates ERK but is also involved in regulation of the Janus-associated kinases

(JAKs) and signal transducers and activators of transcription (STATs).

Like MAPKs, activation of the JAK/STAT cascade can be initiated by paracrine stimulation, although the receptors involved bind cytokine ligands such as cardiotrophin-1 or leukemia inhibitory factor.[73,74] Mechanical stretch of cultured cardiomyocytes or pressure-overload banding both activate the JAK/STAT cascade.[75,76] These factors signal through the common receptor component of the IL-6 family known as gp130, which is involved in cardiomyocyte hypertrophy and survival.[77] Binding of ligands to the gp130 receptor prompts activation of JAK/STAT cascades associated with transcriptional reprogramming of important genes such as bcl-xl and vascular endothelial growth factor.[78] Repression of the JAK/STAT cascade by suppressor of cytokine signaling-3 (SOCS-3) is important for providing the critical counterbalance to maintain appropriate signaling from these powerful hypertrophic pathways.[79] Characterization of crosstalk between activators (ie, gp130) and repressors (ie, SOCS-3) of hypertrophic signaling provides fundamental information regarding how the cardiomyocyte response to hypertrophic signaling is controlled. But cytokine-mediated hypertrophic signaling is a tangled web, as exemplified by leukemia inhibitory factor, which not only promotes hypertrophy via JAK/STAT but also operates through calcium-dependent signaling involving calmodulin-dependent protein kinases and calcineurin.[80] These calcium-activated signaling molecules mediate powerful hypertrophic responses and represent well-studied examples of the necessary homeostatic balance required to maintain cardiac structure and function.

Calcium-Dependent Signaling

Fundamental hemodynamic reciprocity dictates that rising mechanical stress must be countered with increased cardiac contractility. This involves changes in calcium transients. Increases in calcium can involve a variety of mechanisms including stretch-activated channels and L-type calcium channels,[81] synthesis and secretion of natriuretic peptides,[82,83] activation of heat shock factor,[84] and modulation of cell volume.[85] As the mechanotransduction response gathers momentum, combinatorial pathways converge to elevate intracellular calcium levels. Synthesis of vasoactive peptide hormones such as angiotensin II and endothelin-1 prompted by mechanical stretch spread the message as paracrine and autocrine factors to promote calcium release.[86] Endothelin-1 stimulates phospholipase C activity, generating inositol triphosphate and diacylglycerol, two agonists that elevate cytoplasmic free calcium levels.[86–89] Angiotensin II increases intracellular calcium by altering L-type calcium channel conductance and producing capacitive calcium entry, a process wherein depletion of sarcoplasmic reticulum calcium stores elicits a sustained rise in cytosolic calcium dependent on extracellular calcium influx.[90]

In addition to the functional impact of increasing contractility, increasing intracellular calcium levels also activate molecular signaling that ultimately can result in transcriptional reprogramming and structural remodeling. Prolonged calcium-dependent signaling leads to hypertrophy with characteristic changes in cell size, protein synthesis, myocardial mass, and hemodynamic performance. Calcium-activated signaling and the concomitant recruitment of hypertrophic pathways are a critical driving force for the structural remodeling that characterizes hypertrophy. Cardiomyocytes decipher the ambient cytosolic calcium level using calmodulin that, when bound to calcium, triggers the activation of hypertrophic signaling. Calmodulin overexpression induces pathological hypertrophy in vivo,[91] and calmodulin antagonist can block hypertrophy of cultured cardiomyocytes.[92] Calmodulin directs hypertrophy through downstream target molecules such as Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and calcineurin.

The CaMK family consists of multiple members that seem rather promiscuous in substrate preference, phosphorylating a variety of critical functional molecules that regulate calcium levels, contractility, and transcription.[93] Not surprisingly, the expression of activated CaMK induces hypertrophic changes both in vitro and in vivo. Cardiac-restricted expression of CaMK leads to involvement of phosphatase molecules that form complexes with CaMK and are likely to be contributory participants in furthering hypertrophy.[94] Thus, phosphatases represent another branch of Ca^{2+}/calmodulin-dependent signaling integrated with hypertrophic signaling.

Among cardiac phosphatases, calcineurin is the most extensively characterized hypertrophic signaling molecule. Like CaMK, calcineurin is activated by Ca^{2+}/calmodulin-dependent signaling, and cardiac-specific overexpression leads to pathological hypertrophic remodeling.[95] Although the ability to blunt hypertrophic remodeling by inhibition of calcineurin was initially controversial,[96] a preponderance of published evidence indicates that calcineurin is activated by a wide range of stimuli and that inhibition of calcineurin blunts hypertrophic remodeling.[97] In the context of calcineurin-driven hypertrophy, the phosphorylation state of nuclear factor of activated T cells (NFAT) is the key. Dephosphorylated NFAT accumulates in the nucleus and mediates transcriptional reprogramming, leading to hypertrophy.[98] Nuclear accumulation of NFAT is countered by the action of glycogen synthase kinase 3β, which phosphorylates NFAT, thereby increasing nuclear exit of the transcription factor and antagonizing hypertrophic reprogramming.[99]

Additional facets of calcium-dependent signaling in response to mechanical stretch include pathways radiating from pyk2/RAFTK as well as protein kinase C activation. Activation of pyk2/RAFTK, a calcium-activated tyrosine kinase related to focal adhesion kinase (FAK), occurs in response to elevation of intracellular calcium level in cardiomyocytes or hypertrophic stimulation[100] and mechanical stress induced by pressure overload.[101] The activation of pyk2/RAFTK that accompanies cardiac remodeling phosphorylates the adaptor protein paxillin that is a structural component of focal adhesions.[102]

Unlike the involvement of pyk2/RAFTK signaling in hypertrophic remodeling that is only now beginning to be examined, more than a decade has passed since initial observations that implicated protein kinase C activation in stretch-induced hypertrophy.[103] Phospholipase activation in response to mechanical stretch or hypertrophic agonists was reported more than a decade ago.[104] Phospholipase activation

has been implicated in maladaptive signaling leading to heart failure[105] as well as apoptosis in cultured cardiomyocytes,[106] but the consequences of phospholipase activation are probably determined by multipath signaling interactions, because elevation of phospholipase was temporally disconnected from remodeling in vivo.[107] The subsequent generation of diacylglycerol activates members of the protein kinase C family that mediate hypertrophic remodeling.[108,109] More recently, elevation of calcium levels associated with activation of protein kinase C was found to precede development of cardiac hypertrophy.[110] Protein kinase C expression induces hypertrophic remodeling through inextricable connections to the MAPK cascade that foster hypertrophic reprogramming[111] as well as apoptosis.[112]

The Cytoskeleton

The membrane cytoskeleton is a critical junction for signal transduction in cardiomyocytes. The arrangement of the cytoskeleton is perhaps analogous to the ECM insofar as there are a variety of structural and soluble components that are essential for maintenance of cell structure and function. At this interface between cell and extracellular environment, signals are transduced, attachments to matrix proteins are established, and maintenance of cellular shape and structure is preserved. The cytoskeleton similarly provides an intracellular structure for transmitting contractile forces out of the cell to the matrix as well as a pathway for transmitting external forces into the cell and the nucleus and for distributing intracellular loads.

Accumulating experimental and clinical findings show association of cytoskeletal gene mutations with dilated cardiomyopathy.[113] The diversity of these mutations (involving components of the sarcomeric cytoskeleton [titin], the sarcolemmal cytoskeleton [eg, α, β, and δ-sarcoglycan[114]], the z-disk [eg, muscle LIM protein[115]], intercalated disks [vinculin[116]], and the intermediate filament system [desmin[117,118]]) has led to the hypothesis that heart failure may be caused in this setting by an impairment of inside-out or outside-in mechanical signaling, although distinguishing these mechanisms is inherently difficult. This, in turn, would also suggest that similar mechanisms may be involved in acquired forms of heart disease triggered by altered mechanical loading of the heart.

A related possibility is the transmission of stretch-induced changes to alter transcriptional activity by direct coupling of cytoskeletal elements to chromatin or by nuclear trafficking of cytoskeletal protein. The former possibility was hypothesized for intermediate filaments of cardiomyocytes, which form a desmin-lamin network associated with nuclear envelope chromatin that could act to transmit altered forces.[119] In the latter case, muscle LIM protein normally associated with the cytoskeleton[120] accumulated in the nucleus after pressure overload.[121] Whether this redistribution is causal or an epiphenomenon remains unclear, but direct communication between the cytoskeleton and the nucleus remains a tantalizing possibility to explain transcriptional reprogramming in response to stretch.

Cytoskeletal reorganization is a characteristic response of many cell types to fluid shear stress or stretch in vitro.[122,123] Regulation of cytoskeletal structure in many cell types depends on Rho family small GTP-binding proteins that are stimulated through G protein–coupled receptors responsive to agonists released in response to stretch such as endothelin-1, phenylephrine, or angiotensin II.[124] Two family members in particular, rhoA and rac1, are involved in hypertrophic remodeling,[125–128] although both proteins originally achieved notoriety as regulatory switches controlling actin cytoskeletal organization in fibroblasts. Cytoskeletal reorganization is intrinsic to hypertrophic remodeling, so rhoA and rac1 are likely candidates to promote cytoskeletal assembly as well as activate kinases that are implicated in hypertrophic remodeling.[129,130]

The signaling hot spot for the cytoskeleton is the focal adhesion complex: a multimolecular structure consisting of structural proteins, signaling molecules, and transmembrane receptors. Focal adhesions are dynamic structures that respond to mechanical stress with rapid reorganization and formation.[131,132] Integrins play a critical role in directing the assembly of adhesion complexes[133,134] as well as stimulating hypertrophic signaling in response to mechanical stress.[135] Integrin engagement may serve as the stimulus for cytoskeletal remodeling in response to mechanical strain induced by pressure overload in vivo.[133] Upon stimulation, bidirectional multicomponent signaling converges on the focal adhesion complex involving kinases as well as adapter proteins that serve as docking sites for recruitment of additional molecules.[135] Among these molecular participants, FAK is the best characterized member of the cytoskeletal signaling cascade in cardiomyocytes. FAK activation in response to signals provided by the extracellular matrix as well as hypertrophic agonists promotes remodeling.[136] The hypertrophic action of FAK depends, in part, upon association with p130Cas, an adaptor protein localized in adhesion complexes.[137] Additional kinases recruited into the signaling response to stretch include src and phosphoinositide 3-kinase (PI3-K).[138] Involvement of kinases such as PI3-K and FAK in response to mechanical stretch points toward another signaling intersection where cytoskeletal remodeling and cell survival meet. Inhibition of FAK activity in cultured cardiomyocytes leads to loss of adhesion complex structure ultimately ending in cell death by anoikis,[139] indicating the impact of FAK activity on multiple facets of cell structure and function. Whether this process of cell suicide by detachment occurs in the context of heart failure is an emerging issue in ongoing studies of myocardial apoptosis.

Survival

Although the hypertrophic response to stretch is often considered primarily in the context of structural remodeling, activation of survival pathways is equally critical in the molecular compensatory response to stretch. Mechanical stress in the myocardium is an indication that homeostatic conditions have gone awry, and ensuing remodeling combines altered adhesion and elevation of intracellular calcium that could act as a prescription for programmed cardiomyocyte cell death.[139,140] Activation of survival signaling counterbalances proapoptotic stimuli, impinging on the cardiomyocyte, and diminishes cell dropout during the remodeling

process. Hypertrophic and survival signaling often go hand-in-hand as exemplified by calcineurin[141] and IGF-1[142] (which may be linked[143]), TNF-α,[144] cardiotrophin 1,[145,146] and MAPK.[147,148] However, antiapoptotic activities are highly context-dependent and some of these same effectors are capable of contributing to apoptosis under different conditions.[149]

A recurring theme in survival signaling for many hypertrophic cascades involves the PI3-K/Akt pathway. Akt, well known as an antiapoptotic kinase in cardiomyocytes,[150] induces hypertrophic remodeling in vitro and in vivo when overexpressed in a constitutively activated form.[151,152] Elevated mechanical stress induced by pressure overload activates the PI3-K/Akt signaling pathway,[153] perhaps mediated through FAK association with the p85 PI3-K subunit.[154] Modulation of Akt by integrins suggests that matrix-derived mechanical stimulation may be a factor in activity,[155,156] although this possibility remains to be explored in the context of cardiomyocytes. PI3-K/Akt activity is also stimulated through part of the gp130 signaling cascade activated by the paracrine factor LIF.[157,158] Akt is a rather promiscuous kinase, so localization may provide important clues regarding its mode of biological action. Localization of activated Akt in cardiomyocytes suggests that relevant target molecules are nuclear-related.[159] For example, induction of hypoxia-inducible factor 1α (HIF-1α), a cellular transcription factor that regulates VEGF synthesis, is driven by the Akt signaling pathway in response to stretch.[160] Akt-mediated phosphorylation also inactivates glycogen synthase kinase 3β, which exerts powerful antihypertrophic effects in cardiomyocytes.[99,161] Also, a growing body of evidence indicates that Akt influences cardiac growth via activation of the mammalian target of rapamycin (mTOR).[152,162] The actions of Akt are likely to influence and be influenced by concomitant signaling from other hypertrophic pathways.[163] Despite our modest understanding of the mechanisms responsible for integrating these concurrent signals, it is clear that cardiac remodeling in response to mechanical stretch involves signals for cell survival as well as remodeling.

None of the Above: Additional Parallel and Intersecting Pathways

As if the preceding sections are not enough, there is even more. A few additional signals in response to mechanical stretch are featured in an online data supplement (see http://www.circresaha.org), without particular order and with apologies to anyone who feels his or her favorite mechanotransduction pathway has been slighted by omission. This supplemental section briefly highlights heat shock proteins, nuclear factor-κB (NF-κB), *iex-1*, atrial natriuretic factor (ANF), and estrogen.

Conclusions

Although much is known about the individual components of mechanical signaling, it is apparent that much is to be learned concerning the integration of these components. How the mechanical force generated by the contraction of myocytes and fibroblasts influences the organization of the ECM is not known but clearly is important, especially in response to hypertrophic growth as well as in remodeling. Abnormal accumulation and arrangement of the ECM in response to increased mechanical tension would likely lead to abnormal mechanical properties of the ECM and contribute to the overall altered efficiency of the heart. As details of biochemical signaling pathways governing myocardial hypertrophy and remodeling come into more complete focus, our understanding of how these mechanisms apply in vivo will require a corresponding detailed mapping of the physical interactions between the myofilaments, cytoskeleton, membrane, receptors, cell-cell junctions, and ECM components, and how changes in each component redistribute mechanical signals among the other elements of the network.

The cornucopia of mechanical signals impinging on a cardiomyocyte contains powerful activators of the hypertrophic program with profound consequences for myocardial structure and function. The abundance of crosstalk and synergism existing between hypertrophic signaling cascades remains elusive. Moreover, the true magic of adaptive signaling lies in understanding how these multiple bidirectional signals are interrelated into a controlled and balanced remodeling process. Blunted hypertrophic remodeling by selective inhibition of target molecules that seem overtly unrelated should serve as reminders that hypertrophy is an exquisitely integrated process involving numerous components, both critical and/or redundant in nature. Our sophisticated efforts to understand molecular signaling through surgical interventions, transgenesis, or gene deletion are relatively clumsy manipulations compared with the dynamic and subtle shifts in activity that occur in physiological responses. The paradoxical and aberrant effects observed in experimental model systems are likely due to alteration of normal homeostatic regulation resulting from overloading or eliminating a normal facet of the signaling cascade. And yet, these heavy-handed approaches have elucidated and immeasurably enhanced our understanding and appreciation for the complexity of hypertrophic remodeling. Because uncertainties remain regarding the adaptive value of this compensatory response,[164] we must understand how the response to mechanical stress is initiated and controlled. And, as multiplex signaling relationships in the myocardium begin to emerge, the challenge will be to unravel how the myocardium interprets this barrage of information to direct an adaptive remodeling response. And yet, despite the complex orchestrated response of the myocardium, the long-term consequences of remodeling often lead the heart into failure. As functional performance of the stretched myocardium sinks, the accompanying song of signaling molecules, much like the dance band on the *Titanic*, plays on.

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