Costameres, focal adhesions, and cardiomyocyte mechanotransduction

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Samarel, Allen M. Costameres, focal adhesions, and cardiomyocyte mechanotransduction. Am J Physiol Heart Circ Physiol 289: H2291–H2301, 2005; doi: 10.1152/ajpheart.00749.2005.—Mechanotransduction refers to the cellular mechanisms by which load-bearing cells sense physical forces, transduce the forces into biochemical signals, and generate appropriate responses leading to alterations in cellular structure and function. Physical forces encountered by living cells include membrane stretch, gain and loss of adhesion, and compression due to an increase in pressure. The signal transduction pathways that are activated in response to mechanical forces include many unique components, as well as elements shared by other signaling pathways. Mechanotransduction in cardiomyocytes is particularly complex, in that individual muscle cells both respond to externally applied mechanical forces as well as generate internal loads that are transmitted to adjacent cells and their surrounding extracellular matrix (ECM). Mechanotransduction in both atrial and ventricular cardiomyocytes affects the beat-to-beat regulation of cardiac performance but also profoundly affects the proliferation, differentiation, growth, and survival of the cellular components that comprise the human myocardium. Understanding the cellular and molecular basis for mechanotransduction is therefore important to our overall understanding of cardiac structure and function in the normal and diseased heart.

Observational studies conducted during the 1970s addressing the hypertrophic growth response of human myocardium to pathological changes in systolic and diastolic wall stress (42) fostered the development of experimental model systems with which to explore cardiomyocyte mechanotransduction in vivo and in vitro. These model systems now span the breadth of experimental cardiology, from complex large animal models of human cardiovascular disease, to studies conducted on isolated cardiomyocyte membranes subjected to mechanical deformation in vitro. Many studies have relied on highly enriched cultures of isolated cardiomyocytes to identify potential mechanosensors and to define their downstream effectors and molecular targets. Research has also been greatly aided by molecular techniques to overexpress and inhibit specific components of the putative mechanomechanical signaling pathways by gene targeting and related approaches conducted in isolated cells and transgenic animals. These experimental approaches have provided significant insight into identifying the putative subcellular structures responsible for sensing mechanical forces and transducing mechanical signals into biochemical signals that affect cardiomyocyte structure and function. In this brief review, I focus on experimental data indicating that the costamere and its related structure the focal adhesion complex are critical cytoskeletal elements involved in bidirectional mechanomechanical signal transduction in cardiomyocytes.

COSTAMERE STRUCTURE AND FUNCTION

The term “costamere” was first used by Pardo and colleagues (83, 84) to describe vinculin-containing, rib-like bands that encircle the cardiomyocyte perpendicular to its long axis. Resembling the metal ribs of a wooden barrel, costameres flank the Z lines and overlie the I bands of the immediately subjacent sarcomeres. In adult cardiomyocytes in vivo, the costamere consists of a complex protein network that forms a physical attachment of the underlying, outer Z discs of the muscle cell to its surrounding, three-dimensional, stress-tolerant ECM (Fig. 1A). In addition to vinculin, a growing list of other cytoskeletal proteins and signaling kinases have been localized to costameres of striated muscle, thereby providing a direct physical linkage via protein-protein interactions between the...
contractile apparatus within individual muscle cells and ECM proteins within the cardiac interstitium (35) (Fig. 1B).

In addition to their role in cell attachment, Danowski et al. (25) first demonstrated that costameres were sites where contractile forces generated within the cardiomyocyte are directly transmitted to the surrounding ECM. They showed that cultured adult rat ventricular myocytes maintained on a flexible, silicone membrane generated pleat-like wrinkles of the surrounding extracellular matrix each time the firmly attached cells contracted. The pleats were spaced 1.8–2.0 μm apart, coinciding with the distribution of actinin-containing Z discs, and sites of close approximation of the cell membrane to the silicone substratum as determined by interference-reflection microscopy. Costameres are also sites where longitudinal displacement of the ECM is transmitted directly to the contractile machinery of the cell. A 10% static, linear stretch of aligned neonatal rat ventricular myocytes maintained on laminin-coated, microtextured silicone membranes resulted in an immediate, uniform increase in sarcomere length of 10% throughout the entire length of the longitudinally oriented, rod-shaped cell (69). In this case, cell attachment via circumferentially organized costameres alone was sufficient to transmit the externally applied strain directly to the underlying myofibrils, indicating that both externally applied and intrinsically generated mechanical loads are transmitted bidirectionally through costameres.

COSTAMERE-Z DISC INTERACTIONS

Although costameres link the outermost Z discs to the sarcolemmal membrane, internal Z discs also share common features that suggest that they, too, function in mechanotransduction. In general, internal Z discs are rich in cytoskeletal proteins that are involved in actin filament assembly, including the actin capping protein CapZ and α-actinin. α-Actinin cross-links actin filaments within Z discs and is responsible for reversing their polarity between adjacent sarcomeres. CapZ binds to the barbed end of individual actin polymers, thereby terminating the filaments and providing additional anchoring sites for the actin cytoskeleton (93). Both proteins have structural correlates within focal adhesion complexes of nonmuscle cells, and thus in some sense internal Z discs can be regarded as internalized costameres. Their linkage to the sarcolemmal membrane, however, is accomplished indirectly via desmin filament connections with outer Z discs and costameres. Thus intrinsically generated and externally applied mechanical forces are transmitted bidirectionally to the most internally situated sarcomeres of the rod-shaped cardiomyocyte.

FOCAL ADHESION COMPLEXES IN CULTURED CARDIOMYOCYTES

As discussed further below, costameres share many of the structural features of cell-to-matrix adherens junctions and thus
may be considered a striated muscle-specific elaboration of focal adhesions found in cultured nonmuscle cells (35). As techniques were developed to isolate and maintain neonatal and adult cardiomyocytes in long-term culture, it became apparent that cardiomyocytes remodel their adhesive structures to adapt to the two-dimensional culture environment (49, 114). This adaptation includes the reorganization of costameric structures as the cardiomyocytes attach and spread over their ECM-coated substratum, yielding adhesive structures that are structurally similar to typical focal adhesions found in non-muscle cells (27). Immunolocalization studies have confirmed that many, if not all, of the same proteins that comprise the costamere eventually reassemble within focal adhesions as cardiomyocytes attach and spread in culture. In cultured cardiomyocytes that retain contractile activity, or are stimulated to contract in culture, focal adhesion proteins also reassemble along the cell-substratum interface in register with overlying Z discs of the remodeled sarcomeres. These basal, costameric attachment sites, as well as the remodeled adherens junctions derived from the remaining components of the intercalated disc (114), provide strong cell adhesion sites that retain mechanical communication between the ECM and the contractile machinery of the cultured cell.

Focal adhesions are typically flat, elongated structures that form at the ends of actin filament bundles (40) (Fig. 1C). Like their costameric counterparts in vivo, cardiomyocyte focal adhesions contain vinculin and other cytoskeletal proteins that form a dense adhesion plaque at sites of close approximation of the sarcolemma to the ECM (112). A detailed structural analysis of the various focal adhesion components is not yet attainable, but the list of potential protein-protein interactions within focal adhesions would suggest that the molecular architecture is extremely complex. For instance, vinculin can interact with at least 10 other focal adhesion components, including actin, tensin, paxillin, talin, α-actinin, vinexin, and ponsin (142), and many of these binding partners can physically interact with additional components.

Focal adhesion assembly is in part mediated by the mechanical forces placed on the cell, as both stretch and contractility increase the number and size of cardiomyocyte focal adhesions (112, 114). The modulation of focal adhesion assembly/disassembly in response to mechanical load may be related to a primary role for focal adhesion assembly in myofibrillogenesis. Dabiri and colleagues (24) demonstrated that premyofibrils (the precursors of mature myofibrils) form de novo near the cell membrane at sites of focal adhesion formation adjacent to the leading edge of spreading cells. Similarly, Sharp et al. (112) showed that the reassembly of myofibrils following recovery from contractile arrest of neonatal rat ventricular myocytes was preceded by the formation of focal adhesions and costameres containing vinculin and β1-integrins, suggesting an important role for these structures in the earliest steps in myofibrillogenesis.

INTEGRINS LINK ECM COMPONENTS TO COSTAMERIC PROTEIN NETWORK

Integrins are essential components of the bidirectional communication of mechanical forces between the cardiomyocyte cytoskeleton and the ECM (121). Integrins are heterodimeric, integral membrane proteins consisting of single α- and β-chains. At least 18α- and 8β-subunits have been identified, with more than 24 paired integrin receptors expressed on various cell types (98). Additional complexity arises from the existence of multiple isoforms of individual α- and β-chains generated by alternative splicing of α- and β-integrin heterogeneous nuclear RNA (hnRNA) transcripts. Cardiomyocytes express α1, α2, α3, α5, α6, α7, α9, and α10-subunits, whereas the predominant β1-subunit expressed is the ubiquitous β1A- subunit and the striated muscle-specific β1D-isoform (111). The relative expression of the various α-chains varies throughout development, accounting in large part for the differences in adhesive properties of immature versus adult cardiomyocytes. For instance, neonatal rat ventricular myocytes express integrins that predominantly bind to fibronectin and Type I collagen, whereas adult rat ventricular cells express predominantly laminin receptors (126). Integrin receptor subtypes also change in response to hemodynamic overload, suggesting an important regulatory role of integrins in mechanotransduction (4, 126).

Cardiomyocyte integrins are not randomly distributed on the cell surface but rather are found embedded within the sarcolemmal membrane directly adjacent to costameres (121). Thus the ECM-integrin-costameric protein network can be viewed as a highly specialized form of lateral adherens junction in which cell-to-matrix attachment is mediated by direct interaction of cardiomyocyte integrins with specific ECM proteins within the cardiac interstitium. The physical interaction between integrin cytoplasmic domains and adaptor proteins within the cytoskeleton generate a submembrane adhesion plaque that appears critical for transmitting mechanical force between the ECM and the actin cytoskeleton. Furthermore, the cytoplasmic domain of β-integrin subunits plays a direct role in these connections, because at least four cytoskeletal proteins (talin, α-actinin, filamin, and tensin) can link integrins directly to actin filaments (10). With the inclusion of additional protein-protein interactions, it is apparent that integrins provide a critical transmembrane linkage between the cardiac ECM and the actin-based cardiomyocyte cytoskeleton.

INTEGRIN-DEPENDENT SIGNAL TRANSDUCTION AND CELL SURVIVAL

In addition to their structural role, integrin-associated costameres (and their focal adhesion counterparts in cultured cardiomyocytes) are also sites for the localization of signaling molecules that are important in cardiomyocyte survival and growth. These include protein tyrosine kinases such as focal adhesion kinase (FAK), proline-rich tyrosine kinase 2 (PYK2), Src, and c-Src-kinase (Csk), and serine-threonine protein kinases such as integrin-linked kinase (ILK), protein kinase Cε (PKCe), and p21-activated kinase (PAK). Other adaptor proteins, such as paxillin, Crk, DOCK180, and Crk-associated substrate (Cas), can link focal adhesion proteins to other downstream signaling cascades that may be important in both mechanotransduction and growth factor signaling. In general, there appears to be substantial cross talk between integrins and growth factor receptors in many cell types, suggesting that integrin- and growth factor-mediated cellular responses are locally coordinated within focal adhesions (34).

Whereas adhesion of cardiomyocytes has long been considered an important factor in regulating their growth and survival (22), the responsible molecular mechanisms are only now
being identified. Many cell types, including cardiomyocytes, normally require integrin-mediated adhesion to stay alive, and the downstream signals resulting from integrin engagement and clustering within focal adhesion complexes cooperate with those from growth factor receptors to prevent apoptosis (133). Apoptosis mediated by the loss of cell attachment to the ECM is referred to as “anoikis” from the Greek word for “homelessness.” This form of apoptosis contributes to normal tissue homeostasis by ensuring that cells remain in their proper tissue environment. Although the significance of anoikis to human cardiac development and disease is presently unknown, Ding et al. (28) have suggested that anoikis may be a contributing factor to disease progression in some forms of cardiac hypertrophy and heart failure.

Engagement and clustering of β-integrins at the cell surface generate important signaling events that promote cell survival and inhibit apoptosis. Major signaling pathways activated during integrin engagement include Ras and its major effectors phosphoinositide-3 kinase (PI3K) and raf (39). When activated, downstream signaling to Akt (70) and extracellular signal-regulated kinases (ERKs) (5), respectively, promote cardiomyocyte cell survival and inhibit apoptosis induced by variety of noxious stimuli in cardiomyocytes, as well as in and other cells (44).

Recent studies involving overexpression and gene targeting have highlighted the importance of integrins to cardiomyocyte differentiation and survival. Epidermal growth factor (EGF)-mediated proliferation of human fetal myocytes requires the coordinated expression of ECM proteins as well as β1-integrins (52). Transfection of the ubiquitously expressed β1A-isoform, or the muscle-specific β1D-isoform, augmented atrial natriuretic factor (ANF) and myosin regulatory light chain 2v (MLC2v) promoter activity in neonatal rat ventricular myocytes (99). Conversely, overexpression of β1A or β1D cytoplasmic domains inhibited ANF expression and sarcomeric assembly in cultured cells (89, 99) and reduced basal contractility and relaxation in the intact heart (56).

In contrast to aforementioned overexpression studies, homozygous deletion of the β1-integrin gene in mice resulted in early embryonic lethality at E5.5, owing to gastrulation defects soon after implantation (36, 118). However, using embryonic stem (ES) cells derived from these β1-integrin knockout mice, Fassler et al. (37) showed that the differentiation of β1-integrin null ES cells into cardiomyocytes was severely impaired. Although contractile proteins were expressed, their organization into sarcomeres was prevented by the absence of functional β1-integrin heterodimers. Ventricular myocyte-specific excision of the β1-integrin gene using the MLC2v promoter-Cre-LoxP system provided Shai et al. (111) an elegant experimental approach to evaluate integrin function in the intact heart in vivo and in vitro. This approach allows for some cardiomyocyte integrin expression throughout embryonic and adult life, resulting in relatively normal cardiac development and overall survival through adulthood. However, they demonstrated that an ~80% reduction in β1-integrin expression impaired cardiac function and reduced the ability of the heart to withstand pressure overload produced by transverse aortic banding (111). Tissue fibrosis was increased, but interestingly, cardiomyocyte apoptosis was not enhanced, suggesting that the dilated cardiomyopathy that ultimately developed in 6-mo-old animals resulted in part from the loss of myocyte membrane integrity and cardiomyocyte necrosis, rather than apoptosis.

**ECM-INTEGRIN-COSTAMERIC PROTEIN COMPLEX IS A MECHANOSENSOR**

Attachment is clearly one way in which costameres and focal adhesions contribute to mechanotransduction, but there is also substantial evidence to indicate that mechanical forces (generated by passive stretch and active tension development of cardiomyocytes) are “sensed” by costameres and focal adhesion complexes and are transduced into biochemical signals leading to sarcomeric assembly and altered gene expression (Fig. 2). This form of “outside-in” signaling is the predominant mechanism responsible for adaptive growth of cardiomyocytes in response to changing hemodynamic loads. Stretch-induced deformation of cardiomyocyte integrins triggers the recruitment and activation of several signaling kinases, including FAK, Src, Rho-associated coiled-coil containing protein kinase (ROCK), PKCe, and mitogen-activated protein kinases (MAPKs), to the cytoplasmic face of the focal adhesion complex, where they participate in downstream signaling to the nucleus and other organelles (69, 109, 128, 129). Results obtained in mechanically stressed cultured cells complement studies performed in pressure-overloaded, intact myocardium (8, 30, 62, 63, 128) and also support the close interaction between the ECM-integrin-costameric protein complex and growth factor receptor signaling during cardiomyocyte hypertrophy (32, 46, 47, 60, 89, 99, 125).

Exactly how the ECM-integrin-cytoskeletal complex senses mechanical stimuli remains somewhat of a mystery. Seminal observations by Ingber and colleagues (132) using a magnetic twisting device to transfer force directly from integrins to the local cytoskeleton suggests that mechanical deformation of one or more adhesion plaque proteins is the proximal step in an intracellular signaling cascade that leads to global cytoskeletal rearrangements and mechanotransduction at multiple, distant sites within the cell.

FAK is one candidate protein that may be responsible for integrin-mediated mechanotransduction within focal adhesions.
and costameres. FAK is a ubiquitously expressed, 125-kDa nonreceptor protein tyrosine kinase that consists of an NH2-terminal Four.1 protein, Ezrin, Radixin, Moesin (FERM) domain, a central catalytic domain, COOH-terminal proline-rich regions, and a focal adhesion targeting (FAT) sequence (74). The FERM domain may have an autoinhibitory function that maintains the kinase in an unphosphorylated, inactive state (23). The COOH-terminal FAT sequence binds directly to the cytoskeletal adaptor protein paxillin (45) and to talin, a cytoskeletal protein that binds to the cytoplasmic tail of β1-integrins during ECM engagement and integrin clustering (122). Cooper et al. (23) have proposed a two-step model for activation in which FAK binding to paxillin, talin, and other focal adhesion proteins via its FAT sequence produces a conformational change leading to displacement of the FERM domain, thereby releasing its autoinhibition. Once localized, FAK then phosphorylates itself at a single tyrosine residue (Y925) (106). This autophosphorylation site serves as a high-affinity binding site (pYAEI motif) for the SH2 domain of Src-family protein tyrosine kinases. Once bound to FAK, Src then tyrosine phosphorylates FAK at residues Y576 and Y577 within the catalytic domain (which augments FAK kinase activity toward exogenous substrates) and at residues Y861 and Y925 near its COOH-terminus (17). The Y925 phosphorylation site promotes the binding to FAK of Grb2 and other adaptor proteins and kinases containing SH2 domains. The FAK-Src complex also directly tyrosine phosphorylates paxillin (105) and other cytoskeletal proteins involved in focal adhesion and stress fiber formation.

FAK is highly expressed in neonatal and adult cardiomyocytes, and several groups have demonstrated that it undergoes autophosphorylation at Y925 in response to mechanical loading (31, 109, 128). Activation may depend on the redistribution of inactive FAK bound to the COOH-terminal, coiled-coil region of myosin heavy chain to costameres on imposition of mechanical stress (38). However, FAK also undergoes translocation and activation in response to agonists that activate Gq-coupled receptors (1, 6, 32, 46, 47, 60, 88, 99, 125) and receptor tyrosine kinases such as KDR/Flik-1 (123). Thus stretch-induced release of angiotensin II, endothelin-1, or vascular endothelial growth factor from cardiomyocytes and/or nonmuscle cells may also indirectly activate FAK in cardiomyocytes.

Recently, Torsoni et al. (129) addressed the potential mechanisms responsible for FAK activation in response to cyclic stretch of cultured neonatal rat ventricular myocytes. They showed that cyclic stretch-induced FAK activation was highly dependent on the upstream activation of Rho/ROCK, perhaps mediated via local alterations in the actin cytoskeleton at or near sites of integrin clustering. Heidkamp et al. (47) demonstrated that a similar pathway regulates cardiomyocyte FAK activation in response to endothelin-1, which increases Rho-GTP loading in cardiomyocytes (21). However, cyclic stretch-induced FAK activation was not dependent on autocrine/paracrine release of angiotensin II, endothelin, or other growth factors into the culture medium (128), suggesting a more direct effect of integrin engagement and clustering on FAK activation in cardiomyocytes. Indeed, overexpression of FAK-related nonkinase (FRNK), the autonomously expressed COOH-terminal domain of FAK, displaced FAK from cardiomyocyte focal adhesions (46) and also prevented stretch-induced VEGF secretion into the culture medium (139), thus implicating FAK and other focal adhesion proteins as proximal components of the mechanosensory apparatus that mediates stretch-induced growth factor release.

In addition to FAK, cardiomyocytes express a structurally related kinase known as PYK2 [also known as cell adhesion β (CAK-β), related adhesion focal tyrosine kinase (RAFTK), or cell adhesion tyrosine kinase (CADTK)] (3). PYK2 is a Ca2+-dependent nonreceptor protein tyrosine kinase that undergoes bimolecular transphosphorylation (85) in response to integrin engagement, increased intracellular Ca2+, and activation of PKCs in many cell types, including cardiomyocytes (6, 7, 48, 50, 59, 72, 73). Although PYK2 is predominantly localized to the cytoplasm (6), a minor component of the enzyme colocalizes with paxillin in focal adhesions of cultured neonatal rat ventricular myocytes (48). Like FAK, PYK2 acts as an important scaffolding protein and transduces signals from G protein-coupled receptors to downstream MAPK signaling pathways depending on which signaling kinases and adaptor proteins bind to the phosphorylated enzyme (12, 82). PYK2 has also been shown to link a variety of stressful stimuli, including Ca2+ overload, UV irradiation, and tumor necrosis factor-α (TNF-α) treatment to MAPK activation in several cell types (127). Recently, Hirotani et al. (50) demonstrated that PYK2 is an essential signaling component in endothelin- and phenylephrine-induced cardiomyocyte hypertrophy, perhaps acting via the Ca2+- and/or PKC-dependent activation of Rac1.

Bayer et al. (8) demonstrated that PYK2 expression and phosphorylation were significantly increased in adult rat ventricular myocytes in vivo in response to acute left ventricular pressure overload. Similarly, Melendez et al. (73) showed that PYK2 expression and phosphorylation were increased in a mouse model of dilated cardiomyopathy, but its exact role in these conditions has not been elucidated. Nevertheless, recent studies have confirmed that PYK2 is an important upstream regulator of the stress-activated protein kinases (p38MAPK and JNK1/2) in cardiomyocytes (48, 72). Thus PYK2 activation has been implicated in hypertrophic gene expression changes during pathological cardiomyocyte hypertrophy (48) and in the induction of apoptosis (72).

Integrin-linked kinase (ILK) is a third protein kinase that may be involved in integrin-dependent mechanotransduction in cardiomyocytes. ILK is a serine-threonine protein kinase that directly interacts via its COOH-terminal domain with the cytoplasmic tail of β1-integrins (43), as well as other focal adhesion adaptor proteins (138). One of these interacting proteins, PINCH1, is essential to early embryonic development, but appears dispensable when its expression is specifically reduced in cardiomyocytes (65). Very little is known about its function in cardiomyocyte mechanotransduction, but ILK appears to serve both structural and catalytic roles in integrin-dependent signaling in other cell types. ILK is an important upstream regulator of Akt phosphorylation at S473 (87), which is essential for Akt activity, and which may explain the ability of ILK to suppress anoikis (2). ILK interacts with thymosin-β4, an actin-binding peptide that stimulates cardiomyocyte and endothelial cell migration. ILK activation by expression of thymosin-β4 leads to activation of Akt and improved cardiomyocyte cell survival following coronary artery ligation, further implicating ILK and the focal adhesion complex in integrin-dependent cell survival signaling (13).
Nevertheless, it remains unknown whether ILK undergoes translocation and activation in response to mechanical loading of cardiomyocytes in a manner similar to FAK and PYK2.

There is also some evidence to indicate that PKCe, the major novel PKC isoenzyme expressed in cardiomyocytes, is directly involved in integrin-dependent mechanotransduction. Using standard immunofluorescent microscopy, Disatnik et al. (29) first localized PKCe in a striated pattern within myofibrillar structures of cultured neonatal rat cardiomyocytes after stimulation with norepinephrine or phorbol myristate acetate. Subsequently, Huang et al. (54) demonstrated that PKCe translocated in response to arachidonic acid treatment of adult rat ventricular myocytes to a region adjacent to the Z line where actin filaments are anchored and where transverse tubules are closely apposed to the myofilaments. This site of translocation was specific for PKCe as PKCα, (the other novel PKC expressed in rat ventricular myocytes) translocated to the nucleus in response to arachidonic acid. Borg et al. (14) then showed that PKCe localized to the cytoplasmic side of the sarcolemma directly adjacent to the Z disc, and Heidkamp et al. (47) demonstrated that the kinase colocalizes with FAK in typical focal adhesions in cultured neonatal cardiomyocytes. These morphological results are complemented by biochemical data demonstrating that PKCe forms functional signaling complexes with PYK2 (92) and Src-family protein kinases (91, 117, 130) in tissue homogenates of left ventricular myocardium from PKC-overexpressing mice. PKCe, in turn, is involved in the endothelin-induced activation of both FAK (47) and PYK2 (7) via signaling pathways that may regulate local changes in the actin cytoskeleton. Thus there is ample evidence to indicate that at least a portion of cardiomyocyte PKCe is found in costameres and focal adhesions, where it may regulate focal adhesion and costamere formation (119) and sarcomeric assembly (69) in response to mechanical loading and growth factor stimulation.

One way that PKCe may localize to costameres and focal adhesions is via binding to receptor for activated C-kinase-1 (RACK1). RACK1 is a seven-trypotphan-aspartate (WD)-domain-containing protein that binds to the cytoplasmic tail of β-integrins (67) and anchors PKC isoenzymes (76), Src family protein kinases (18), and other proteins to focal adhesions. Although originally described as a selective receptor for activated, Ca\(^{2+}\)-dependent PKCs (107), Besson et al. (11) demonstrated that RACK1 also binds active PKCe and increases focal adhesion formation, integrin clustering, and lamellipodia formation in human glioma cells. These responses are quite similar to those produced by overexpression of constitutively active PKCe in cultured neonatal cardiomyocytes (119). Once localized, PKCe can phosphorylate a number of membrane-anchored and cytoskeletal proteins and participate in the activation of Rac1, which is required for cell spreading and cytoskeletal assembly (9). Alternatively, active PKCe binds directly to the Z disc (95) via an interaction with a RACK2-like protein (53), creating a situation in which PKCe may locally shuttle between costameres and Z discs to regulate contractile function in response to changing hemodynamic loads.

It remains unknown exactly how PKCe is locally activated in response to mechanical loading. Vuori and Ruoslahti (131) showed that PKC activity in the cell membrane fraction transiently increases preceding cell spreading on fibronectin but not on polylysine, and PKC activation is required for FAK activation in response to cell spreading on fibronectin. These results would suggest that a membrane phospholipase within integrin-dependent cell attachment sites provides a local source of diacylglycerol sufficient to activate PKCe. Phospholipase C (PLC)-γ can bind to the Y\(_{397}\) phosphorylation site of FAK (86), and Ruwhof et al. (101) have shown that PLC (but not phospholipase D) activity rapidly increases in neonatal cardiomyocytes in response to cyclic stretch, suggesting that PKC activation may be both upstream and downstream of FAK activation in response to mechanical loading.

**OTHER MUSCLE-SPECIFIC, INTEGRIN-BINDING PROTEINS, AND CARDIOMYOCYTE MECHANOTRANSDUCTION**

Melusin is a striated muscle-specific, cysteine-rich, 38-kDa protein that is also localized to cardiomyocyte costameres (16). The COOH-terminal tail region of the molecule directly interacts with the cytoplasmic tail of β\(_1\)-integrins, where it appears to function as part of the mechanosensory apparatus that transduces increased pressure (produced by aortic constriction) to gene expression changes resulting in the progression of left ventricular hypertrophy to heart failure (15, 26). Melusin-null mice develop normally but demonstrate cardiac dilatation rather than concentric left ventricular hypertrophy in response to transverse aortic banding (15). Interestingly, the animals still demonstrate a normal concentric, hypertrophic growth response to infusion of subpressor concentrations of phenylephrine and angiotensin II, suggesting that melusin is not necessary for cardiac hypertrophy induced by neurohormonal stimuli that do not involve mechanical stress. In contrast, overexpression of melusin protects the heart from the development of eccentric hypertrophy and heart failure in response to long-standing pressure overload (26).

These exciting results are reminiscent of studies involving the Z disc adaptor muscle LIM protein (MLP), which is required for the induction of specific features of cardiomyocyte hypertrophy in response to stretch but not Gq-coupled receptor activation (58). MLP is a member of a large family of proteins that contains one or more double zinc finger structures (LIM domains) mediating specific contacts between proteins that participate in the formation of multiprotein complexes (137). Mice deficient in MLP (MLP\(^{-/-}\) mice) developed a progressive cardiomyopathy characterized by LV chamber dilatation, decreased ejection fraction, and premature death from congestive heart failure (51). Isolated papillary muscles from juvenile MLP\(^{-/-}\) mice have increased passive stretch properties, suggesting an intrinsic abnormality in titin function. Importantly, neonatal cardiomyocytes isolated from MLP\(^{-/-}\) mice failed to increase brain natriuretic peptide and ANF mRNA levels in response to 10% biaxial passive stretch but respond normally to Gq-coupled receptor stimulation with phenylephrine, indicating a specific defect in mechanotransduction. MLP interacts with multiple proteins within the Z disc, including α-actinin and the titin-binding protein telethonin (T-cap). Absence of MLP causes the displacement of T-cap in a small minority of cardiomyocytes, suggesting that the loss of MLP leads to destabilization of the anchoring of the Z disc to the proximal end of the T-cap/titin complex, thereby affecting the role of titin in mechanical stretch receptor function.

Exactly how the loss of MLP interferes with downstream signals for cardiomyocyte survival and hypertrophy remains
and p38MAPK), cell survival pathways that involve PI3K and activated in response to mechanical loading. These include the diomyocytes, and many different downstream effectors are growth factor-dependent signal transduction pathways in cardiac muscle cells (57).

MECHANOTRANSDUCTION AND GROWTH FACTOR SIGNALING

As discussed above, engagement and clustering of integrins at the sarcolemmal membrane directly generates a variety of biochemical signals that affect cardiomyocyte structure and function. However, mechanical loading also causes the local release of peptide growth factors and cytokines that can interact with their cell surface receptors on cardiomyocytes and nonmuscle cells, and thereby indirectly affect cardiac performance (120). Obviously, this autocrine/paracrine signaling still must require one or more mechanosensors to interpret changes in mechanical load and provide appropriate intracellular signals that increase growth factor transcription, translation, and secretion. Since the first description of the rapid release of preformed angiotensin II into the culture medium of cultured neonatal rat ventricular myocytes subjected to static stretch (103), several studies have documented that mechanical load causes the increased expression and release of a variety of factors, including angiotensin II (75, 103, 113), endothelin-1 (141), VEGF (108), TGF-β (100), and cardiotrophin-1 (81) by cultured neonatal cardiomyocytes. There is also some evidence to suggest that acute mechanical loading rapidly releases similar growth factors and cytokines from adult cardiomyocytes (20, 64). However, few studies have addressed the responsible mechanisms involved in transducing mechanical signals into biochemical signals that regulate growth factor expression and secretion by cardiomyocytes.

Another consequence of mechanical loading is the transactivation of growth factor-receptor tyrosine kinases (104). Transactivation may occur by autocrine-paracrine release of angiotensin II or endothelin-1, activation of their cognate receptors, which is then followed by intracellular tyrosine phosphorylation of the EGF receptor (59). Alternatively, receptor tyrosine kinases may undergo more direct activation (and in the absence of exogenous agonist) following integrin engagement and clustering, wherein integrin-dependent FAK, PYK2, Src, and/or ILK activation and intracellular transphosphorylation of the receptor plays an intermediary role. A third possibility involves the autocrine/paracrine release by cardiomyocytes or cardiac fibroblasts of matrix metalloproteinases in response to mechanical loading. The metalloproteinases, in turn, release heparin-binding growth factors such as HB-EGF from the ECM and thereby stimulate downstream signaling (110).

CONSEQUENCES OF MECHANOTRANSDUCTION IN CARDIOMYOCYTES

A complex signaling web connects mechanosensory and growth factor-dependent signal transduction pathways in cardiomyocytes, and many different downstream effectors are activated in response to mechanical loading. These include the well-described, MAPK signaling cascades (i.e., ERKs, JNKs, and p38MAPK), cell survival pathways that involve PI3K and Akt, the Janus kinase/signal transducers and activators of transcription (JAK-STAT) signaling pathways, and Ca²⁺/calmodulin-dependent pathways involved in the activation of calcineurin and calmodulin-dependent protein kinases. The processing of most mechanosensory signals is likely to involve the ECM-integrin-costameric protein complex, although other mechanosensors within the sarcolemmal membrane and the Z disc may also contribute (57). Mechanotransduction via integrins and their accessory nonreceptor protein kinases resembles the downstream signals generated following activation by neurohormonal agonists, and these interconnecting signaling pathways have been extensively reviewed in the recent literature (77, 97, 102, 120, 121). Thus mechanosensory pathways share critical components with signaling pathways activated in response to norepinephrine, endothelin-1, angiotensin II, and other peptide growth factors and cytokines. These responses include acute alterations in contractile function, as well as long-term, structural changes resulting from an increased rate of cardiomyocyte protein synthesis (including the contractile proteins myosin and actin), enhanced sarcomeric protein assembly, and a generalized decrease in the susceptibility of sarcomeric proteins to intracellular proteolysis (Fig. 3). In response to certain pathological forms of hemodynamic overload, mechanosensory pathways also increase the transcription rates of cardiomyocyte-specific genes that are normally expressed predominantly during fetal life (i.e., activation of the “hypertrophic” or “fetal” gene program) (90). Reactivation of the fetal gene program improves energy utilization in the face of reduced myocardial flow reserve but also alters the contractile properties of the adult myocardium resulting in reduced force generation, abnormal calcium handling, and slowed myocardial relaxation. Mechanosensory pathways also affect cell shape and normal cell-to-cell communication (113, 134, 143) and produce local alterations in the actin cytoskeleton that affect ion channel gating and responsiveness to β-adrenergic receptor signaling (19, 68, 135, 136). Finally, there is some evidence to suggest that mechanical overload of cardiomyocytes can directly or indirectly lead to the induction of apoptosis (55, 66).

![Fig. 3. Consequences of cardiomyocyte mechanotransduction. Mechanosensory signaling pathways activated during outside-in signaling modulate multiple cardiomyocyte functions.](http://ajpheart.physiology.org/)

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SUMMARY AND FUTURE DIRECTIONS

It should be evident from this brief review that there has been substantial progress during the past decade in characterizing the role of costameres and focal adhesions in cardiomyocyte mechanotransduction. The accumulated evidence strongly implicates these structures as important components of the mechanosensory apparatus of heart muscle cells, although other structural components may also contribute. Indeed, stretch-activated ion channels and stretch sensors within the myofilaments, the Z discs, and the intercalated discs may also contribute to sense mechanical stimuli and to transmit biochemical signals to distant sites within the cell. Nevertheless, it has become apparent that the ECM-integrin-costameric protein complex has taken center stage in the search for the elusive cardiomyocyte “stretch receptor” involved in regulating cardiomyocyte growth, atrophy, and survival in response to varying hemodynamic loads (61).

Despite considerable progress, several important issues need to be addressed regarding the roles of costameres and focal adhesions in cardiomyocyte mechanotransduction. First, better model systems are required to ensure that results obtained using cultured cardiomyocytes faithfully reproduce the effects of mechanical load on cardiomyocytes in the intact heart. Most of the results reviewed above were obtained using neonatal rat ventricular myocytes in serum-free, two-dimensional culture, and there are several instances where experiments conducted using these immature cells do not reflect the responses of adult cardiomyocytes in vivo. Furthermore, isolated neonatal cardiomyocytes maintained in two-dimensional culture form randomly oriented, cell-matrix attachments only at their bottom surface. This adaptation to the two-dimensional culture environment differs significantly from the circumferential, costameric attachments produced by rod-shaped adult cardiomyocytes in vivo.

Second, mechanical load varies depending on the fiber orientation within the tissue. Although there have been some attempts to examine the differential effects of cell orientation (i.e., random vs. aligned) and the direction of stretch (i.e., parallel vs. perpendicular to the long axis of aligned cardiomyocytes) on mechanochanical signaling (41, 78, 79, 115, 116), it remains unclear whether costameres are required for cell signaling in either or both directions. It is conceivable that different mechanosensors are required to sense transverse versus longitudinal stretch, perhaps accounting for differential signaling and cellular phenotype resulting from pressure versus volume overload.

Third, mechanical load varies throughout the cardiac cycle, and a complex relationship exists between the internal load generated during excitation-contraction coupling and external load generated during chamber filling and fiber shortening of adjacent cardiomyocytes. An elegant study by Yamamoto et al. (140) highlights the critical importance of the timing of externally applied strain to specific phases of the cardiac cycle, and similar studies are needed to define the interactions between excitation-contraction coupling events, externally applied mechanical loads, and cell signaling via costameres as well as other mechanosensors.

Fourth, there likely exists a complex, bidirectional interplay between mechanotransduction occurring in cardiomyocytes and their supporting nonmuscle cells (i.e., endothelial cells and fibroblasts). However, most mechanotransduction studies have been confined to relatively pure populations of either isolated cardiomyocytes or nonmuscle cells. As investigators develop methods to assemble more complex, three-dimensional scaffolds for tissue engineering (94), it may be possible to dissect the relative contribution of the nonmuscle cell population to cardiomyocyte mechanotransduction under conditions that are more representative of the in vivo environment.

Finally, there are substantial structural differences between atrial and ventricular myocytes, but there have been relatively few studies that have analyzed costamere and focal adhesion function in atrial cells (124, 135, 136). However, atrial fibrosis and cardiomyocyte ECM interactions have long been implicated in the pathogenesis of atrial fibrillation and other conditions affecting atrial function (80), and additional studies seem warranted. These and other issues should keep experimental cardiologists interested in mechanotransduction very busy over the next several years.

GRANTS

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REFERENCES


