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Mechanotransduction in Cardiac Hypertrophy and Failure

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Abstract

Cardiac muscle cells have an intrinsic ability to sense and respond to mechanical load through a process known as mechanotransduction. In the heart, this process involves the conversion of mechanical stimuli into biochemical events that induce changes in myocardial structure and function. Mechanotransduction and its downstream effects function initially as adaptive responses that serve as compensatory mechanisms during adaptation to the initial load. However, under prolonged and abnormal loading conditions, the remodeling processes can become maladaptive, leading to altered physiological function and the development of pathological cardiac hypertrophy and heart failure. Although the mechanisms underlying mechanotransduction are far from being fully elucidated, human and mouse genetic studies have highlighted various cytoskeletal and sarcolemmal structures in cardiac myocytes as the likely candidates for load transducers, based on their link to signaling molecules and architectural components important in disease pathogenesis. In this review, we summarize recent developments that have uncovered specific protein complexes linked to mechanotransduction and mechanotransmission within (1) the sarcomere, (2) the intercalated disc, and (3) at the sarcolemma. The protein structures acting as mechanotransducers are the first step in the process that drives physiological and pathological cardiac hypertrophy and remodeling, as well as the transition to heart failure, and may provide better insights into mechanisms driving mechanotransduction-based diseases.

Keywords

heart; cardiac muscle; mechanotransduction; sarcomere; cytoskeleton; cardiac hypertrophy/remodeling; heart failure; genetically altered mice; sarcolemma; cell-cell junction

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DISCLOSURES

None.

INTRODUCTION

In cardiac muscle, a number of proteins have been proposed as key mechanosensors and mechanotransducers that directly sense and respond to mechanical loads (stress and/or strain) triggering structural, signaling and functional alterations associated with various cellular processes. These processes can include regulation of electrophysiology via stretch-sensitive channels, myocardial material properties and fibrosis, contractile function and calcium regulation, and the important downstream effect of mechanical signaling: cardiac muscle growth via hypertrophy and atrophy. In addition to transducing a mechanical signal, proteins and complexes can act as structural transmission conduits for mechanical stress and strain (mechanotransmission). For example, transferring stress generated at the actin-myosin complex, through the sarcomere, Z-disc and cytoskeleton, to the sarcolemma. These structural proteins can thus be critical components of a mechanotransduction pathway. This review will summarize evidence for mechanotransduction at three key sites within the cardiac myocyte/cytoskeleton: the sarcomere, the intercalated disc and the sarcolemma.

An abundance of evidence points to the sarcomere as a key site for mechanotransduction. The sarcomere is the basic contractile unit of cardiac muscle, and is made up of a complex assembly of myofilament proteins (1). The interaction between myosin heads (thick filament) and actin thin filament proteins is the key process that drives force-generation in cardiac muscle (2). In addition to their role in force generation, the direct connection of the cytoskeleton to the sarcomeric Z-disc and myosin's stabilization by titin and other structural proteins, suggest that forces can be transmitted in and out of the sarcomere, reinforcing the idea that sarcomeric proteins play a role in mechanotransduction (3, 4). Growing evidence points to specific complexes within the sarcomeric Z-disc as well as within the I band (linked to titin) in sensing and responding to mechanical loads driving downstream events including those that regulate gene expression, protein synthesis and degradation within cardiac muscle cells (1). Mechanical forces generated by the sarcomere are transmitted both longitudinally and laterally to the sarcolemma of the cardiac muscle cell (5), as there is evidence for directional-dependence of myocyte stress sensing (6, 7), hence there may be directional dependence of mechanotransduction within a myocyte. Such directional dependence in load sensing may be related to different modes of hypertrophic growth (8).

In addition to the complex protein structures within the sarcomere and intracellular cytoskeleton, the intercalated disc and sarcolemma at the outer boundaries of the cardiac myocyte are possible key sites for transduction of mechanical forces. Mechanical force or stress can be transmitted both outside-in and inside-out through the membrane components of a cell. Since the internal cytoskeleton is coupled via transmembrane proteins to the extracellular matrix (ECM) (9) and hence the rest of the myocardium, membrane-associated protein complexes have been widely investigated as sites regulating mechanotransduction. In terms of lateral connections, the sarcomere connects to the sarcolemma by cytoskeletal components that link the Z-disc with the membrane-spanning integrin and dystroglycan complexes, which then bind to components of the ECM (9). The intermediate filament desmin also connects the Z-disc to the nucleus (10). On the other hand, longitudinal connections also take place between cytoskeletal actin and intermediate filaments with

fascia adherens and desmosomal junctions, respectively, both of which are components of the specialized cardiac muscle cell-cell junction, the intercalated disc (11).

Although the mechanisms underlying mechanotransduction in cardiac muscle are not known, putative proteins and regional complexes have been suggested to be involved in this highly sensitive and critical regulatory pathway within the cardiac myocyte. These structural connections provide a conduit in which to further explore and uncover protein complexes and signals that impact the cellular response to mechanical cues involved in cardiac hypertrophy, as well as calcium/contractile function (12), regulation of fibroblasts and material properties (13), and electrophysiology (14). Dysregulation in these functional processes can lead to an altered cellular response to external forces and possibly the transition to heart disease.

(1) SARCOMERE-MEDIATED MECHANOTRANSDUCTION

The sarcomere is the molecular motor of the cardiac myocyte, and force generated by actin-myosin interactions are transmitted in a complex fashion through the sarcomere itself, and then to the rest of the cytoskeleton, sarcolemma and eventually through the ECM to the surrounding myocardium. As forces from outside of the cell are transmitted back through the structural proteins to the sarcomere, several sarcomere-level molecules could be involved with load sensing and transduction of both internal and externally generated forces (**Figure 1**). Titin and muscle LIM protein (MLP, also known as cysteine rich protein 3 (CSRP3)) are likely two critical players in force transmission and sensing within the sarcomere. Both proteins have a vast network of interacting proteins, and form complex signalosomes that have been implicated in mechanotransduction pathways and hypertrophy-related signaling (15, 16).

Titin-Directed Mechanotransduction—Titin is a giant sarcomeric protein that spans from the Z-disc to the M-line (17). Multiple roles for titin have been established, which include functioning as a molecular ruler for sarcomere assembly, generating passive stiffness in the sarcomere during stretch as well as serving as signal transducer in response to mechanical overload (18-21). Unique regions within titin have been shown to serve as anchoring sites for a variety of cytoskeletal proteins that orchestrate and possibly transmit biomechanical stress responses during cardiac hypertrophy and failure. These proteins include MLP, titin-Cap (TCAP), calsarcin-1 (CS-1), four-and-half-LIM domain protein-1 (FHL1) and muscle-specific ankyrin repeat protein (MARF) family members that have all been implicated to play important roles in titin directed mechanotransduction (22).

MLP/TCAP/Titin Complex—It has been proposed that the Z-disc MLP/TCAP/titin complex functions as a stretch sensor in cardiac muscle cells, and that defects in the complex can lead to cardiomyopathy and heart failure development (16). MLP is composed of two LIM domains and is localized to the Z-disc through direct binding to α -actinin (23, 24). Cardiac muscles cells deficient in MLP respond to treatment with the hypertrophic agent endothelin-1 (ET-1) with a characteristic upregulation of the fetal gene markers, including atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) (16). The

absence of this fetal gene response in MLP-deficient cells exposed to mechanical stress (16) suggests that MLP may play a specific role in mechanosensing and signaling.

MLP knockout mice display a severe dilated cardiomyopathy phenotype resulting in heart failure and premature death (25). A common dilated cardiomyopathy-associated polymorphism found in human MLP (W4R-MLP), resides within the TCAP binding region of MLP and has been shown to abolish binding of TCAP to MLP (16). It has been proposed that loss of this MLP/TCAP interaction induces abnormal intrinsic elastic properties of titin resulting in the inability of cardiac muscle cells to properly sense mechanical stress (16). In support of this concept, a MLP^{W4R/W4R} knock-in mouse model generated by Knöll and colleagues displayed hypertrophic cardiomyopathy and developed a heart failure phenotype (26). The observation of ventricular hypertrophy in these mice in the absence of pressure overload suggests that the W4R-MLP polymorphism impairs mechanotransduction signaling in response to basal levels of load. Mutant hearts displayed reduced MLP mRNA and protein levels, as well as increased nuclear localization of W4R-MLP. Furthermore, *in vitro* studies demonstrated reduced binding of TCAP to W4R-MLP, which could suggest a plausible mechanism for the translocation of W4R-MLP from the Z-disc into the nucleus. However, the role of MLP within the nucleus of cardiac muscle cells has yet to be clearly defined. MLP does not have the ability to bind DNA directly, but following the discovery of cysteine rich protein 2 (CSR2), a protein closely related to MLP (MLP is also known as CSR3), which could enhance gene expression in smooth muscle cells through binding to GATA and serum response factor (SRF) transcription factors (27), there reigns the possibility that MLP may have the ability to bind and modulate important cardiac transcription factors such as GATA-4 and SRF in cardiac muscle cells in response to mechanical stimulation. The discovery that the potassium channel β -subunit minK interacts with TCAP at the sarcomere, suggests that TCAP may also serve to link myofibrillar components to the sarcolemma. Although not fully explored, it has been proposed that this interaction could potentially connect titin deformation with potassium influx in cardiac muscle (28). Equally as important, with the emergence of evidence suggesting that MLP has a predominantly cytosolic localization (29), and studies demonstrating that the titin-TCAP complex is a rigid superstable complex at the Z-disc that is optimized to resist applied loads (30), is the need for further research to assess whether MLP-TCAP is in fact a direct mechanosensor complex that has the required capacity to undergo conformational changes under physiological forces.

MARP/Titin Complex—MARP family members, which include three members; cardiac ankyrin repeat protein (CARP), ankyrin repeat domain protein 2 (ANKRD2) and diabetes related ankyrin repeat protein (DARP) have all been shown to interact at the N2A region of titin and proposed to serve as a stress response signalosome (31). Miller and colleagues demonstrated that rat cardiac muscle cells respond to passive stretch by initiating both an increase in and redistribution of MARP proteins, which included translocation of CARP and DARP into the nucleus (31). Given the fact that CARP has been shown to act as a negative regulator of cardiac gene expression (32), the authors proposed that this redistribution of MARPs into the nucleus in response to myofibrillar stress/strain is an example of stretch-based sensing and signaling (31). In support of the concept of increased MARPs in response

to stress, it was found that CARP and ANKRD2 gene expression is increased in response to a single bout of eccentric contractions in the mouse (33). CARP has been shown to be upregulated in heart failure (34, 35), hypertrophy (36), and mutations in the ANKRD1 gene encoding CARP have been shown to be causative for human dilated and hypertrophic cardiomyopathy (37-39). The latter of these studies showed in rat embryonic myocardial cells *in vitro* that human ANKRD1 mutations can lead to differential stretch-induced gene expression when compared with controls (39), providing further evidence for a role for CARP in stretch sensing. However, recently generated MARP triple knockout mice, have cast doubt on the importance of MARP proteins for cardiac function (40). Under basal conditions and in response to biomechanical stress induced by mechanical pressure overload for 14 days, the mice were found to be viable with normal cardiac function (40), suggesting that compensatory mechanisms may counteract the global loss of MARPs. Therefore, it would be interesting to examine the effects of cardiac specific loss of MARPs at postnatal stages in response to biomechanical stress. Other potential reasons for these contrasting results could arise from both species differences (rat vs. mouse) and/or the mode of stimulation used (cyclic stretch vs. TAC). Hence it would be interesting to examine whether MARP triple KO mice display a cardiac phenotype after extended periods of TAC (greater than 14 days), or in response to other cardiac stress and injuries (e.g., myocardial infarction, etc.). Recent studies have also highlighted a role for MARP proteins in signaling pathways relating to PKC and PKA, thus looking at mouse models deficient in these signaling proteins under mechanical stress may be a useful research avenue to explore (41).

FHL1/Titin Complex—The titin N2B region resides within the extensible I band region of titin, which contributes to the myofibrillar passive/diastolic tension generated upon stretch (42). FHL1 has been shown to bind to titin at this region (43), and has been shown to be upregulated in mouse models in response to both pressure overload induced hypertrophy and treatment with hypertrophic agents (44, 45). This upregulation has also been shown to occur in the hearts of human patients exhibiting hypertrophic cardiomyopathy (46-48), together suggesting that FHL1 may play an important role in biomechanical stress responses in cardiac hypertrophy and heart failure. In support of, and extending upon this idea, we showed FHL1 to be part of a biochemical stress sensing signalosome that scaffolds components of the mitogen activated protein kinase signaling pathway (RAF1 (rapid accelerated fibrosarcoma-1)/ MEK2 (mitogen-activated protein kinase kinase-2)/ ERK2 (extracellular regulated kinase-2)), specifically at the N2B stretch sensor domain of titin (43). These studies showed that mouse hearts devoid of FHL1 did not develop a basal cardiac phenotype but demonstrated a blunted hypertrophic response and preserved LV function in response to pressure overload, which was associated with loss of ERK2 phosphorylation (43). Interestingly, FHL1 deficient cardiac muscles also exhibited an increase in cardiac muscle compliance, consistent with loss of function of the titin N2B stretch sensor domain (43). Given the importance of G α q (Gq) pathways in pressure overload-induced hypertrophy (49), we further sought to cross FHL1 deficient mice with the transgenic mouse model harboring constitutively active Gq overexpression (43). The results suggest a direct link between FHL1-mediated mechanotransduction pathways and Gq signaling pathways as FHL1 deficiency was shown to prevent the cardiomyopathy and pathological ERK2 phosphorylation, caused by constitutively active Gq overexpression (43).

Recent studies showed that FHL1 deficient mice crossed with a hypertrophic cardiomyopathy mouse model (MHC^{403/+}) resulted in increased cardiac hypertrophy (50), suggesting that there may be distinct stress-induced hypertrophic signaling cascades mediated by FHL1. Further mechanistic studies by revealed that FHL1 is a negative regulator of titin N2B phosphorylation (51). We recently showed that titin N2B is a novel substrate of ERK2 and FHL1, and directly interferes with ERK2-mediated titin-N2B phosphorylation (51). Together our results suggest the working hypothesis that FHL1 restricts titin based cardiac muscle compliance by masking/interfering with kinases (ERK2) that bind to titin N2B in order to limit titin N2B phosphorylation and compliance to “physiological ranges” with stretch (51). It may also act as a scaffold for MAPK-mediated hypertrophic signaling (51). However, loss of FHL1 may render titin N2B sites “open” to kinases that bind titin N2B resulting in increased titin N2B phosphorylation and compliance beyond “physiological ranges”, which then destabilize the MAPK scaffold to inactivate hypertrophic signaling (51).

Titin kinase—The M-line-associated region of titin contains a protein kinase domain (52) that has been proposed to play a role in mechanosensation (53). Using single molecule analytical techniques, Puchner and colleagues demonstrated that physiological levels of mechanical stress can initiate the catalytic activity of titin kinase by releasing its active site to allow binding of its co-factor, adenosine triphosphate (54). Mechanically induced titin kinase interacts with a protein complex composed of the autophagosomal receptors, NBR1 (neighbor of BRCA1) and p62, also called sequestosome-1 (SQSTM1), and the E3-ubiquitin ligase, muscle-specific RING finger-2 (MURF2) (55). *In vitro* studies in cardiac muscle cells demonstrated dissociation of this signalosome in response to mechanical inactivity induced by hyperkalemic depolarization, leading to translocation of MURF2 to the nucleus (55). Within the nucleus, MURF2 induces both downregulation and nuclear export of SRF, suggesting that MURF2 acts as a repressor of SRF-dependent gene expression in response to a loss of mechanical loading (55). Conversely, a more recent study has emerged that suggests the kinase domain of titin is in fact a pseudokinase with undetectable levels of catalysis (56). The authors propose that the titin pseudokinase functions as a scaffold that supports the recruitment of proteins to the sarcomeric M-line. This finding, coupled with the fact that MURF2 knock out mice do not display a detectable basal cardiac phenotype (57), highlights the need for additional studies to establish whether the mechanoactivation of titin kinase and subsequent phosphorylation of downstream targets coupled with the NBR1/SQSTM1/MURF pathway regulates cardiac mechanosensing, or whether any potential signaling function of titin via NBR1/SQSTM1/MURF is solely due to a scaffolding role performed by the kinase domain.

An increasing body of literature suggests that SQSTM1 and NBR1 also serve to provide a link between protein ubiquitination and the process of selective autophagy, which is responsible for the regulated removal of proteins and organelles (58). SQSTM1 and NBR1 mediate direct interactions between polyubiquitinated proteins and microtubule-associated protein light chain 3 (LC3)-II, which is required for autophagosome recruitment (59). Recent evidence suggests an emerging role for autophagy in the clearance of signaling proteins (59, 60), which raises the possibility that changes in SQSTM1/NBR1 localization in

response to mechanical stimuli could directly influence autophagic protein turnover and cardiac muscle cell signal transduction pathways. Support for the importance of titin kinase in the regulation of protein turnover via SQSTM1/NBR1 was provided in a human patient with a point mutation in the α R1 helix, R279W (Arg34091Trp) of titin kinase, which abrogates binding to NBR1 (55). This mutation was originally associated with hereditary myopathy with early respiratory failure (HMERF), that is characterized by focal myofibrillar breakdown (61), as well as aberrant localization and aggregation of SQSTM1 (55). More recently, titin domain A150/Fn3 119 has emerged as a hotspot for mutations causing HMERF (62). Indeed, patients harboring the originally identified R279W mutation in the kinase domain were also found to contain a missense mutation (Pro30091Leu) in A150/Fn3 119 *in cis* (63). The controversy whether either one or both mutations are causative of the hereditary myopathy (63-65), and the underlying molecular mechanisms contributing to disease pathology, remain to be further analyzed. However, a recently identified titin kinase-W260R (p.Trp34072Arg) mutation that causes early-onset cardiomyopathy may support the importance of titin kinase for proper muscle function. Indeed, this mutation was also found to abrogate NBR1 binding, thereby further implicating defects in titin kinase to aberrant protein turnover and the development of myopathies (66).

MLP-Calcineurin-NFAT—MLP has also been shown to be essential for anchoring the Ca^{2+} activated phosphatase, calcineurin (CnA) at the Z-disc (24). In response to activation, CnA dephosphorylates the NFAT (nuclear factor of activated T-cells) family of transcription factors, leading to their translocation into the nucleus and the activation of a pro-hypertrophic gene program (67, 68). Studies using MLP heterozygous deficient mice have shown that mislocalization of CnA in response to reduced MLP levels is associated with reduced NFAT signaling, pronounced LV dilatation and cardiac dysfunction after myocardial infarction (24). These findings suggest that MLP has the ability to transduce biochemical stress to the nucleus via CnA-NFAT activation, and that reduced MLP-CnA signaling can lead to adverse remodeling in response to myocardial infarction.

MLP-PICOT—Protein kinase C-interacting cousin of thioredoxin (PICOT) is a protein found at the Z-disc that can inhibit CnA-NFAT hypertrophic signaling in response to hypertrophy induction by phenylephrine (69). PICOT localizes to the Z-disc through an interaction with MLP, which in turn abrogates the binding of CnA to MLP, leading to its displacement from the Z-disc. The displacement of CnA from the Z-disc has been shown to prevent CnA-NFAT activation and thus inhibit cardiac hypertrophy (69). This mechanism has also been postulated to protect against pressure overload-induced cardiac hypertrophy, with NFAT gene upregulation shown to be significantly diminished in the hearts of PICOT-overexpressing transgenic mice exposed to pressure overload (69). However, it has yet to be established whether PICOT binding to MLP at the Z-disc prevents any nuclear translocation events in response to mechanical stimulation.

Calsarcin-1—Calsarcin-1 (CS-1) is an additional Z-disc localized protein that interacts with and negatively regulates calcineurin activity. CS-1 knockout mice display enhanced calcineurin activation, and develop both increased cardiac hypertrophy and an exacerbated cardiomyopathy in response to pressure overload (70). Mutations in CS-1 have been linked

in humans to a form of hypertrophic cardiomyopathy characterized by early onset and cardiac arrhythmias (71).

(2) INTERCALATED DISC-MEDIATED MECHANOTRANSDUCTION

Force developed within the sarcomere is transmitted longitudinally to interdigitated distal ends of the cardiac muscle at specialized junctions known as the intercalated discs, which play a key role in maintaining mechanical and electrical coupling between cardiomyocytes (11). A study performed in rabbit hearts undergoing cyclical volume overload and unload revealed that the intercalated disc undergoes dynamic ultrastructural changes associated with sarcomere assembly/disassembly in response to volume overload, supporting a role for the intercalated disc as a site of mechanotransduction, which is associated with cardiac muscle cell growth and hypertrophic responses (72). The two most prominent structures within the intercalated disc that are thought to sense and process mechanical stress are the fascia adherens and desmosomal junctions, based on their integral links and mechanotransmission to cytoskeletal actin and intermediate filaments, respectively (22) (**Figure 2**).

Fascia adherens junctions—Fascia adherens junctions are anchoring junctions between cells that connect the membrane bound cadherins that span the extracellular space at the junction to cytoskeletal actin filaments to provide strong adhesion between neighboring cells (73). In the heart, the proteins bound to the fascia adherens junctional complex include (i) transmembrane proteins that are mainly composed of N-cadherin (NCAD), which are calcium-dependent, but also include coxsackievirus and adenovirus receptor (CAR) and lysosomal integral protein 2 (LIMP2)), which are then intracellularly linked to (ii) catenins (α (α -CAT), β (β -CAT), $\alpha\text{v}\delta\gamma$ ($\pi\lambda\alpha\kappa\sigma\gamma\lambda\sigma\beta\iota\nu$, JUP)), which regulate cadherin-based activity as well as (ii) catenin binding proteins such as muscle specific mouse Xin alpha ($mXin\alpha$), vinculin(VIN)/metavinculin(MV) and α -actinin, which modulate catenin activity or act to link the fascia adherens junction to cytoskeletal actin (73, 74). Thus, by scaffolding multi-molecular complexes, that include components with known signaling roles (eg., β -CAT) and anchoring the actin cytoskeleton, it is thought that several aspects of mechanotransduction converge at the fascia adherens junction(74, 75).

A mechanotransduction role has been postulated for N-CAD within the fascia adherens junction complex. N-CAD is upregulated in response to applied mechanical stretch(76), and N-CAD–catenin complexes have been shown to transmit mechanical forces by forming attachment sites between adjacent cardiac myofibrils(74). Elegant *in vitro* studies performed by Chopra and colleagues revealed a direct role for N-CAD in cardiac muscle mechanotransduction (76). Specifically, by exploiting cardiomyocytes on a N-CAD-substrate, their studies revealed that N-CAD mediated adhesions were capable of eliciting a cytoskeletal-mediated mechanical remodeling response, which included changes in cardiomyocyte shape, myofibrillar organization and function (as measured by traction forces and cortical stiffness), suggesting the importance of adhesion-contractile balance in cardiac myocyte growth (76). These findings were reinforced when inhibitors of myosin contractility were shown to lower N-CAD-mediated effects on cardiomyocyte stiffness, highlighting an adaptive response of the cardiomyocyte cytoskeleton to changes in mechanical stimuli (76). Interestingly, the spreading and stiffness adaptations of cardiac

muscle cells were more enhanced when N-CAD was engaged as opposed to integrin-based substrates, further suggesting that different adhesion systems may mediate differential cytoskeletal adaptive responses based on the perceived forces (76). These concepts may also have implications in cardiac disease settings where differential cardiomyocyte cell growth responses are observed (e.g, dilated cardiomyopathy-axial cell lengthening (eccentric) versus hypertrophic cardiomyopathy-transverse (concentric) cell growth) (76). Potential evidence to support this comes from studies focused on the specific contribution of N-CAD in the adult heart, which exploited a cardiac-specific and inducible N-CAD knockout mouse model (77). Loss of N-CAD in adult mouse cardiomyocytes resulted in the complete absence of identifiable intercalated disc structures (loss of fascia adherens and desmosomal junctions as well as reduction in levels of the gap junction protein, connexin43), culminating in cardiac morphological and functional defects, associated with a modest form of dilated cardiomyopathy (77). However, unlike typical dilated cardiomyopathy, which reveals an enlargement of left ventricular chambers in the short axis, the enlargement was more pronounced in the long axis(77), highlighting an *in vivo* switch in cardiomyocyte growth response that may be consistent with loss of a mechanotransductive role for N-CAD. Interestingly, adult N-CAD knockout mice also displayed ventricular arrhythmias leading to sudden death (77). N-CAD knockout hearts also displayed decreased sarcomere length and wider but less dense Z-discs, consistent with loss of muscle function due to the absence of N-CAD and anchoring of myofibrils to the plasma membrane(77), which highlight that alterations in cardiomyocyte cell-cell mechanosensing can directly impact sarcomere alignment and protein assembly. Interestingly, increased β -1 integrin levels were also observed in adult N-CAD knockout hearts (77), further highlighting the engagement of a differential cytoskeletal adaptive response associated with integrin-based function. Although a role for N-CAD in human cardiac disease remains to be clarified, these studies suggest a contributing role for N-CAD in multiple mechanotransductive pathways in cardiac muscle.

Alpha-catenins (α -CAT) are key molecules that link the cytoplasmic domain of cadherin to the actin cytoskeleton (78). Recent studies have also implicated a role for α -CAT as a force transducer that is important for mechanotransduction at cadherin-based junctions (79). Specifically, *in vitro* studies by Yonemura and colleagues showed that α -CAT recruits VIN, another main actin-binding protein of the fascia adherens junction, through force-dependent changes in α -CAT conformation that unmask VIN binding sites to promote adherens junction development (79). Interestingly, our group has also demonstrated that α -E-catenin is required for VIN localization to the fascia adherens junction in cardiomyocytes *in vivo* (80). Specifically we showed that adult cardiomyocytes from cardiac-specific α -E-catenin knockout mice exhibited specific loss of VIN at the intercalated disc but not the costamere, highlighting a requirement for the cadherin/catenin/VIN complex at the fascia adherens junction (80). *In vivo* studies focused on the cardiac-specific α -E-catenin knockout mice further revealed that loss of α -E-catenin and resulting VIN loss lead to defects in cardiac intercalated disc structure, morphology and function, associated with a progressive form of dilated cardiomyopathy that encompassed right ventricular wall thinning (80). We also showed that cardiac-specific α -E-catenin knockout mice exhibited an increased propensity to ventricular wall rupture and decreased survival following myocardial infarction at stages prior to disease manifestation (80), highlighting a loss in the cardiomyocyte's ability to adapt

to mechanical load with α -E-catenin deficiency. Similar observations related to increased vulnerability to infarct rupture were observed in transgenic mice harboring a C-terminal truncated α -E-catenin (81). Interestingly, defective expression and localization of α -E-catenin at the intercalated disc is a feature of human patients prone to ventricular rupture following myocardial infarction (81), highlighting the relevance of these mechanotransduction-associated pathways in human cardiac disease. Given the molecular crosstalk between α -E-catenin and VIN at the fascia adherens junction, it is also important to note that cardiac-specific and heterozygous conventional VIN knockout mice also exhibit cardiac intercalated disc and functional abnormalities either at baseline, which were associated with dilated cardiomyopathy or after hemodynamic stress associated with increased susceptibility to pressure (mechanical) overload, respectively (82, 83). Human dilated cardiomyopathy patients with MV mutations also exhibited similar intercalated disc defects to the cardiac-specific α -E-catenin knockout mice (80, 84), highlighting that the α -E-catenin/VIN protein complex harbors signals important in maintaining the structural and functional integrity of the intercalated disc and heart, respectively, that also translate to human disease settings. These studies focused on uncovering a mechanistic link between the cellular alterations and functional deficits associated with VIN deficiency in mice, and uncovered a novel mechanism linked to the role of VIN at the costamere (5), which will be discussed in more detail in the Sarcolemma section.

Biochemistry-based studies have also associated a role for the striated muscle-specific protein, nebulin-related anchoring protein (N-RAP) in cardiac muscle mechanotransduction at sites that intersect between the fascia adherens junction and sarcomere (85, 86). Part of this evidence comes from studies that have identified N-RAP to contain a C-terminal actin-binding domain and N-terminal LIM domain, which highlight mechanosignaling domains within N-RAP (86). A physical association between N-RAP and fascia adherens junctions was revealed when N-RAP was shown to co-purify with actin-based intercalated disc components and the fascia adherens junction fragments positively staining for N-RAP and VIN (86). Interestingly, detergent-stripped intercalated disc fractions, which rendered the fraction devoid of actin and VIN, still showed an association between cellular N-RAP within the intercalated disc fraction (containing, N-CAD, α -actinin, desmin, connexin 43), highlighting that N-RAP's association with the fascia adherens junction is mediated by cadherins (86). Gel overlay assays also revealed an association between N-RAP and α -actinin (86). Although a role for N-RAP has not been determined in cardiac muscle *in vivo*, N-RAP expression was found to be highly upregulated and abnormally distributed in MLP knockout hearts(85), raising the possibility that N-RAP functions may also intersect with the sarcomere via MLP-related mechanotransduction pathways associated with heart failure.

Desmosomes—Desmosomes are anchoring junctions that serve to mechanically couple cells in tissues that undergo constant mechanical stress, such as the heart (11). They tether the cytoskeletal intermediate filament network between cardiomyocytes (11). In cardiac muscle, these organized, disc-shaped, electron-dense structures are composed of extracellular transmembrane-based cadherins, desmocollin-2 (DSC2) and desmoglein-2 (DSG2), which provide a platform via their cytoplasmic tails to members of the armadillo family, JUP and plakophilin-2 (PKP2), that are then in turn bound to the plakin family

member, desmoplakin (DSP), which is the central cytoplasmic link to the load-bearing intermediate filament network composed of desmin (11, 73). A role for desmosomes in cardiac mechanotransduction and long range force transmission across cells stems from its association with the desmin intermediate filaments, which were originally termed as 'mechanical integrators of cellular space' because of connections to various parts of the cell, including the nucleus, mitochondria, desmosome and sarcomeric Z-disc (10). Desmosomes could also be downstream mechanical transducers of fascia adherens junctions as desmosomal assembly and function were shown to be dependent on N-CAD in the adult heart (77). Studies have also identified hybrid junctions known as the area composita that are composed of proteins from both the fascia adherens and desmosomal junctions, highlighting a convergence of pathways (87). However, desmosomal proteins appear to have a distinct and robust role in intermediate filament-based mechanotransduction, based on growing evidence from genetic data that point to a distinct cardiomyocyte remodeling response associated with desmosomal deficiencies and mutations (separate from the fascia adherens junction), that link to the human cardiac disease, arrhythmogenic right ventricular cardiomyopathy (11).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic-based cardiac disease tightly associated with desmosomal abnormalities(88). Recent studies have also highlighted that mutations in the spring element of titin (IG10) cause an ARVC-like disease (89). A connection between titin and the desmosome was recently highlighted as titin filaments have been shown to connect to a new subcellular domain of the intercalated disc, termed the transitional junction (90), highlighting a potential for titin-based sarcomere pathways to intersect with the desmosome and ARVC. Further studies, possibly through the generation of knock-in mouse models, will be required to determine the direct role for these mutations in ARVC and whether these pathways directly intersect with the desmosome. Hallmarks of ARVC include life-threatening arrhythmias, cardiac dilation and dysplasia of one or both ventricles that are worsened by strenuous exercise, as well as cardiomyocyte death and replacement of myocardial tissue with fibro-fatty infiltration. Ventricle contractility is compromised in ARVC hearts with late-stage ARVC culminating into heart failure(88). Mutations in desmosomal components can account for up to 58% of ARVC cases(91). A number of studies have exploited genetic mouse models to reveal an important role for desmosomes in transducing mechanical cues into a wide variety of cellular responses important for cardiac structure, function and disease features reminiscent of human ARVC. Mutations in the desmosomal cadherins, DSC2 and DSG2 are associated with human ARVC (11, 92). Studies carried out in transgenic mice expressing the human ARVC-associated DSG2 mutation (N266S) revealed that within two weeks after birth, transgenic mice developed spontaneous ventricular arrhythmias, conduction slowing, ventricular dilation and aneurysms, fibrosis and calcification, leading to sudden death (93), which was reminiscent of a biventricular form of ARVC. Supporting this study, targeted deletion in mice of the extracellular domain of DSG2, which is a domain likely capable of sensing mechanical loads and known to be important in desmosomal adhesive activity as well as signaling, also leads to a biventricular form of ARVC encompassing upregulation of heart failure markers, fibrosis, biventricular dilatation and dysfunction, and spontaneous death(94). At the ultrastructural level, mouse hearts displayed an enlargement of the

intercellular gap at the intercalated disc associated with the loss of desmosomal structure which seemed to coincide with visible heart lesions at the macroscopical level(95). These studies together highlight a key role for desmosomal cadherins in sensing and responding to mechanical stresses associated with cardiac muscle contraction.

An important cytoplasmic intermediary that interconnects the desmosomal junction to the cytoskeletal-based intermediate filament system is DSP (11). We and others have revealed through the generation of various genetic mouse models harboring mutations and/or loss of DSP (96-98), that DSP is critical for maintaining cardiac desmosomal cell-cell adhesion (intercalated disc) integrity and function. Cardiac-specific loss of DSP in mice *in vivo* (using a ventricular myosin light chain-2 Cre recombinase (MLC2v-Cre) mouse line) resulted in early ultrastructural defects that were associated with loss of desmosomal but not N-CAD based junctional proteins (98). Furthermore, these mice recapitulated the postnatal onset of human ARVC at the histological (cardiomyocyte death, fibrosis, fatty infiltration), physiological (biventricular dysfunction and heart failure) and electrophysiological (arrhythmias and premature death) levels that was also observed in an ARVC patient harboring a recessive DSP mutation (98, 99). DSP knockout mice (using MLC2v-Cre) also exhibited sarcomeric defects including loss and widened Z-discs, consistent with loss of muscle function (98), revealing that alterations in desmosomal cell-cell mechanosensing (load) and adhesion can directly impact sarcomere structure. Interestingly, cardiac-specific transgenic mouse models harboring a human DSP mutation that impacted its binding to intermediate filaments also resulted in a cardiac disease reminiscent of ARVC (97), further highlighting that intermediate filament-based mechanotransduction associated with the desmosome may be linked to the cardiac disease, ARVC.

Several studies have also probed for signaling targets at the desmosome that contribute to the various hallmarks of ARVC. We and others have highlighted that developmental signals that elicit transdifferentiation of cardiac muscle to adipocytes play a contributory role towards the fibrofatty infiltration associated with ARVC (98, 100). Although the mechanisms have not been fully elucidated, loss of JUP from the desmosomal cell-cell junction and resulting loss in Wnt/ β -CAT signaling (due to presence of nuclear JUP or pathogenic Hippo signaling) has been postulated as a hallmark of ARVC (101) and thought to play a role in promoting adipogenic/fibrogenic gene expression in ARVC, respectively (102, 103). A recent study has highlighted a potential direct link between JUP and PKP2 in cardiac muscle shear stress responses (104). Specifically, this study revealed that shear stress can trigger cardiomyocyte junctional remodeling and that neonatal rat ventricular cardiomyocytes overexpressing JUP and PKP2 mutations associated with ARVC displayed an abnormal response to shear stress (104). Expression of the JUP mutant 2057del2 also displayed an increased propensity for cardiomyocyte apoptosis in response to shear stress (104), which was also observed with cyclical stretch (105). Interestingly, stress responses could be reversed by treatment with the glycogen synthase kinase-3- β inhibitor, SB216763 (104, 105), highlighting convergence between mechanical and signaling responses at the desmosome. Studies in genetic mouse models have also revealed a role for JUP in stabilizing mechanical properties of cardiac muscle *in vivo*, which include a distinct desmosomal cellular remodeling response associated with features associated with ARVC.

Aged global heterozygous JUP knockout mice exhibited right ventricular enlargement and slowed conduction associated with spontaneous arrhythmias that were exacerbated with exercise (106). Interestingly, the exercise training-induced ARVC exhibited by these mice could be rescued with load-reducing therapy (loop diuretic furosemide and nitrates) that reduced cardiac ventricular pressure and volume overload (107). Cardiac-specific JUP knockout mediated by alpha myosin heavy chain Cre (α MHC-Cre) largely recapitulated the features of a biventricular form of ARVC, which included progressive biventricular dilation and dysfunction associated with fibrotic replacement of the myocardium, cardiomyocyte death and spontaneous arrhythmias resulting in sudden death in mice as early as two months of age (108). Although sarcomeric structure, fascia adherens junctions and gap junctions were preserved, desmosomal structures were absent from the intercalated disc of JUP knockout hearts (108). Independent studies generating a cardiac-specific plakoglobin knockout model (using tamoxifen inducible α MHC-Mer-Cre-Mer inducible mouse), highlighted similar findings except that no cardiac arrhythmias were observed despite gap junction remodeling and that mice survived longer (109). Interestingly, discrepancies in links to β -CAT signaling were also reported between both cardiac-specific JUP deficient mouse models (108, 109), which have been similarly observed between cardiac-specific DSP deficient mouse models (96, 98), requiring further evaluation. Nonetheless, these studies altogether highlight that an absence of JUP and desmosomes renders cardiomyocytes unable to properly respond to high mechanical stress resulting in myocyte dissociation and a cellular remodeling responses uniquely associated to ARVC. Since increased β -CAT was observed and proposed to be a compensatory response to the loss of JUP in cardiac-specific JUP-deficient mice (108), double cardiac-specific JUP (α MHC-Cre) and β -CAT (using α MHC-Mer-CreMer inducible mouse) knockout mice were generated (110). JUP and β -CAT double knockout mice caused a loss of fascia adherens and desmosomal junctional proteins resulting in mice exhibiting cardiomyopathy, fibrous replacement of the myocardium and strong arrhythmogenic phenotypes, with 100% of them succumbing to sudden death within 3 to 5 months after tamoxifen injection (110). Interestingly, transgenic mouse models overexpressing JUP (wild type or mutant associated with ARVC), also exhibited fibrofatty replacement of the ventricle and higher prevalence of sudden death(102). Thus, it has been suggested that even small levels of JUP overexpression are sufficient to disrupt the mechanical and signaling functions of JUP in cardiac muscle (111).

Loss of the intermediate filament protein desmin in mice *in vivo* also leads to skeletal and cardiac muscle abnormalities (112, 113). Specific to the heart, desmin null mice exhibited cardiomyocyte ultrastructural defects (intercalated disc and sarcomere) and cardiomyocyte death resulting in fibrosis and calcification preferentially in the interventricular septum and right ventricle, which lead to cardiomyocyte hypertrophy, ventricular dilatation and systolic dysfunction (113, 114), reminiscent of ARVC. Human mutations in desmin are also associated with familial forms of skeletal and cardiac myopathies (115), including ARVC (116). Recent studies have revealed that some of these severe disease altering mutations may directly impact the force bearing properties of desmin filaments (eg., overstretching at low forces or premature stiffening at low deformations) resulting in defects in the mechanosensing and transduction abilities of desmin (117).

(3) SARCOLEMMA-MEDIATED MECHANOTRANSDUCTION

Besides the intercalated discs at the ends of cardiac myocytes, sarcolemma-associated proteins and complexes along the lateral surfaces of elongated myocytes have been described as foci of force transmission and mechanotransduction(118). Equivalent to non-muscle focal adhesions, costameres are crucial in the lateral transmission of force from the Z-disc of the sarcomere to the sarcolemma and ECM (118, 119). By forming a connection between the ECM and the contractile apparatus, costameric structures facilitate the maintenance of mechanical integrity of the sarcolemma (119, 120). A complex network of cytoskeletal and signaling proteins converge at these specialized cell junctions, and therefore costameres are likely key mediators of mechanical related signaling, and possibly involved with transducing mechanical signals bi-directionally between the extracellular space and intracellular signaling networks (119) (**Figure 1**).

Integrins—Communication between the ECM and the intracellular portion of cardiac costameres is facilitated by transmembrane integrin receptors attached in the extracellular space to ECM ligands, and intracellularly to the cytoskeleton by proteins such as talin, VIN, and α -actinin(121, 122). Integrin receptors are dimers of α and β subunits, and different combinations of these subunits confer the receptor specificity for ECM ligands(123). The patterns of expression of specific integrin subunits in cardiac muscle cells can be altered and resemble fetal patterns under specific pathologic conditions (124). Specifically, $\alpha 5$ and $\alpha 7$ subunits can be significantly upregulated in ischemia or following myocardial infarction (125), while $\alpha 1$, $\alpha 5$, $\alpha 7$, and $\beta 1 D$ subunits are increased in response to pressure overload (126), suggesting that the myocardium may respond to mechanical stress through the modulation of integrin related signaling molecules. In support of this, dilated cardiomyopathy with extensive myocardial fibrosis and reduced tolerance to loading was observed in a mouse model with excision of $\beta 1$ integrin using the MLC2v-Cre mouse line (127). Furthermore, reduction of $\beta 1$ integrin in adult mouse cardiomyocytes resulted in impaired hypertrophic response to pressure overload as a result of modified adrenergic-mediated signaling downstream of the hypertrophic stress (128). Integrins are also fundamental nodal points of key signaling pathways related to mechanotransduction. Integrin-linked kinase (ILK) is an important transducer of integrin signaling. Mutations in ILK have been reported in human dilated cardiomyopathy (129) and tetralogy of Fallot (130). Mice bearing a cardiac knockout of ILK develop dilated cardiomyopathy and spontaneous heart failure(131). Studies performed in human induced pluripotent stem cell-derived cardiomyocytes indicated that ILK mediated cardiomyocyte force transduction via regulation of the sarcoplasmic/endoplasmic reticulum Ca(2+)-ATPase isoform 2a (SERCA-2a) and phosphorylation of phospholamban (PLN) in the human heart(132). Thus, ILK links mechanoreception to the dynamic modulation of cardiac contractility by interacting with the functional SERCA-2a/PLN axis.

Vinculin-Talin-Integrin Complex—VIN is a cytoskeletal actin-binding protein that links the actin cytoskeleton to the sarcolemma. VIN is specifically tethered at the costamere, where it binds to integrins via talin. Mutations in the muscle-specific isoform of VIN, MV were found in patients with both dilated and hypertrophic cardiomyopathy, highlighting its importance for normal cardiac function (84). The presence of VIN at the intercalated disc,

which is another important site of mechanical coupling, has made it difficult to precisely dissect specific VIN functions between these two cellular regions (124, 133). However, studies in mouse models have demonstrated that VIN is essential for mediating the response to mechanical stress in the heart. Homozygous global loss of VIN in mice leads to embryonic lethality (at embryonic day 10) (134). However, mice with heterozygous loss of VIN display normal basal cardiac function but are more susceptible to cardiac dysfunction and display increased mortality in response to mechanically mediated dysfunction induced by pressure overload of the left ventricle (82).

Talin is a cytoskeletal protein that links integrins to the actin cytoskeleton through the cytoplasmic domain of the β -integrin subunit, and modulates integrin activation and ligand binding (124). Talin mediates the recruitment of key signal transduction partners such as focal adhesion kinase (FAK) and phosphatidylinositol-4-phosphate-5 kinase type I γ to the costameres (135). Studies performed in mice have revealed that talin1 protein expression and targeting to the costameres is increased in response to cardiac hypertrophy induced by pressure overload (119). Furthermore, it was demonstrated that cardiomyocyte-specific knock-out of talin 1 in mice leads to a blunted hypertrophy response and preserved cardiac function in response to pressure overload when compared to littermate controls (119). The results suggested that talin 1 mediates the hypertrophy response to mechanical stress in the heart, and that a reduction of talin1 expression in cardiac muscle cells can lead to improved cardiac remodeling following pressure overload.

In addition to a role at the costameres, FAK and its C-terminal binding partners p130Cas (p130 CRK-associated substrate) and paxillin (PAX) also redistribute to the Z-disc with hypertrophic stress. FAK has been shown to mediate integrin signaling leading to hypertrophy, while also being activated by α -1 adrenergic stimulation (136). Furthermore, in neonatal cardiomyocytes, tyrosine phosphorylation of FAK and p130Cas has been shown to increase upon ET-1 stimulation (a hypertrophic agonist), which also promotes redistribution of FAK, Cas and PAX to sarcomeric Z-discs (137). Cas alone or in cooperation with Src non-receptor tyrosine kinase modulated basal and ET-1-stimulated ANF gene expression. Interaction of Cas with FAK, as well as their localization to Z-discs, appears critical for sarcomeric assembly in cardiac myocytes. Therefore, the assembly of signaling complexes that include the focal adhesion proteins, Cas, FAK and PAX at Z-discs in the cardiac myocytes may regulate, either directly or indirectly, both cytoskeletal organization and gene expression associated with cardiac myocyte hypertrophy (137). Further work indicated that expression of different Cas mutants leads to severe sarcomeric disarray in cardiomyocytes(138). Expression of the C-terminal focal adhesion-targeting domain of FAK disrupted sarcomeric organization and mislocalization of endogenous Cas to Z-discs, suggesting that the association of FAK and Cas as well as preservation of multiple protein-interaction motifs of Cas was required for proper sarcomeric assembly in cardiac myocytes(138). Interestingly, in skeletal muscle, changes in the expression of FAK levels and signaling were also associated with sarcomeric reorganization in response to overload (139).

Caveolin 3—Integrin signaling in response to mechanical load can also be regulated by caveolin 3 (CAV3) (140). CAV3 is highly expressed in cardiac muscle cells, where it can be

found both at the focal adhesion complex(141), or as a component of lipid raft microdomains, termed caveolae (142), that can regulate signal transduction by compartmentalizing receptors and their ligands (143). Loss of CAV3 in cardiac muscle cells has been shown to lead to reduced β 1 integrin expression leading to perturbed basal and stretch mediated signaling responses (140).

Dystroglycan—The dystroglycan complex also serves as an ECM receptor in cardiac muscle cells. β -dystroglycan interacts with α -dystroglycan on the cell surface, which facilitates interactions, with the ECM protein laminin (144). β -dystroglycan also tethers the actin binding protein dystrophin within the cytosol (145). Mutations in dystrophin have been shown to lead to Duchene's muscular dystrophy, which is characterized by muscle (skeletal and cardiac) degeneration (146, 147), highlighting the importance of dystrophin as linker between the ECM and the cytoskeleton. Combinatorial loss of dystrophin with β 1-Integrin has been shown to exacerbate the cardiomyopathic changes observed with loss of dystrophin and integrins alone in response to isoproterenol treatment, suggesting cross talk between these two complexes in response to cardiac stress (148).

CONCLUSIONS/FUTURE DIRECTIONS

Force transmission and sensing within the cardiac myocyte is a complex process, and a multitude of proteins and protein complexes have been implicated in the mechanisms of both stress transmission and stress transduction. The putative mechanotransduction-related proteins exist at many different locations within the cell, from the outer sarcolemmal membrane down to the force-generating sarcomere and associated structures. It appears likely that multiple structural pathways may be involved with the overall processes of load-mediated remodeling of cardiac myocytes, with redundant or overlapping function.

It is intriguing to consider modulation of a mechanosensing pathway in order to alter the downstream effects, for example, adverse remodeling in myocardium could be controlled at the force-sensing level, altering the downstream molecular signals and improving long-term outcomes in cardiac disease. Mediating the molecular signals associated with a specific mechanotransduction pathway could also be a way of modifying downstream remodeling outcomes, without directly changing the structural properties of the force transmission pathways. For example, as described above, several of the hypertrophy-inducing pathways in cardiomyocytes are, at least in part, calcium-dependent: among others, calcium-calmodulin kinase II (CaMKII) and CnA are activated by increased local calcium concentrations. These calcium-mediated signaling pathways have been investigated as mediators of sarcomeric force generation, and are clearly described. Thus small molecule modulations of these well-described pathways are underway and represent a promising direction for therapeutic applications for cardiac hypertrophy and failure (149, 150). Inhibitors of CaMKII have proven excellent research tools and some have been investigated in early clinical trials (151). Amongst those, the CaMKIIN peptide has shown minimal off-target effects, but has yet to be tested in patients (151). CnA inhibitors cyclosporin A (CsA) and FK506 were shown to prevent the phenotypic manifestations of hypertrophic cardiomyopathy in mice overexpressing tropomodulin, or fetal β -tropomyosin (152, 153). Partial or complete inhibition of cardiac hypertrophy has been achieved with CsA and

FK506 following pressure overload in rats and mice (154-156), although it has also been reported that under specific settings a significant rescue could not be achieved (154-157). Studies in rats indicated that CsA prevented exercise-induced cardiac hypertrophy, attenuated hypertrophy and histopathology in double transgenic transgenic mice overexpressing human renin and angiotensinogen (158) as well as attenuated myocardial infarction-induced cardiac hypertrophy (152, 159). CsA also reduced cardiac hypertrophy in constitutively activated Gq transgenic mice (160). Nevertheless the known side effects of CsA and FK506 in humans have prevented further exploration of these small molecules (152). A similar approach could be used for other signaling kinases implicated in mechanotransduction pathways such as ILK, which mediates force transduction in cardiomyocytes by modulating SERCA-2a/PLN function (132). The R211A mutation in ILK has been shown to promote enhanced cardiomyocyte contractility compared to its wild-type counterpart, including increased releasable SR calcium content. Such positive effects were proposed to stem from higher expression levels of ILK and SERCA-2a compared with that resulting from ILK WT overexpression in vitro and in transgenic mice in vivo. Thus, gene therapy with ILK R211A has been proposed as a possible future management strategy for dilated cardiomyopathy (132).

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Nonstandard Abbreviations and Acronyms

α-CAT	α -catenin
αMHC-Cre	α -myosin heavy chain Cre recombinase
αMHC-Mer-Cre-Mer	Tamoxifen inducible α -myosin heavy chain Cre recombinase
β-CAT	β -catenin
ANF	Atrial natriuretic factor
ANKRD1	Ankyrin repeat domain protein 1
ANKRD2	Ankyrin repeat domain protein 2
ARVC	Arrhythmogenic right ventricular cardiomyopathy
BNP	Brain natriuretic peptide
CAR	Coxsackievirus and adenovirus receptor
CARP	Cardiac Ankyrin Repeat Protein
CAMKII	Calcium-calmodulin kinase II
CAV3	Caveolin-3
CnA	Calcineurin

CsA	Cyclosporine A
CS-1	Calsarcin-1
CSR2	Cysteine rich protein 2
CSR3	Cysteine rich protein 3
DARP	Diabetes related ankyrin repeat protein
DSC2	Desmocollin-2
DSG2	Desmoglein-2
DSP	Desmoplakin
ECM	Extracellular Matrix
ET-1	Endothelin-1
ERK2	Extracellular regulated kinase-2
FAK	Focal adhesion kinase
FHL1	Four and a half LIM domain protein-1
Gq	Gq signaling pathway
HMERF	Heredity myopathy with early respiratory failure
ILK	Integrin linked kinase
JUP	γ -catenin (plakoglobin)
LC3-II	Microtubule-associated protein light chain 3-II
LIMP2	Lysosomal integral protein 2
MARP	Muscle-specific Ankyrin Repeat protein
MEK2	Mitogen-activated protein kinase kinase-2
MLC2v-Cre	Ventricular myosin light chain-2 Cre recombinase
MLP	Muscle LIM protein
MURF2	Muscle-specific RING finger-2
MV	Metavinculin
NBR1	Neighbor to BRCA1
N-CAD	N-cadherin
NFAT	Nuclear factor of activated T-cells
NRAP	Nebulin-related anchoring protein
p62/SQSTM1	p62/sequestosome-1
p130Cas	p130 CRK-associated substrate
PAX	Paxillin

PICOT	Protein kinase C-interacting cousin of thioredoxin
PKP2	Plakophilin-2
PLN	Phospholamban
RAF1	Rapid accelerated fibrosarcoma-1
SERCA-2a	Sarcoplasmic/endoplasmic reticulum Ca(2+)ATPase isoform 2a
SRF	Serum Response Factor
TCAP	Titin-Cap
VIN	Vinculin
W4R-MLP	Dilated cardiomyopathy-associated polymorphism found in human MLP

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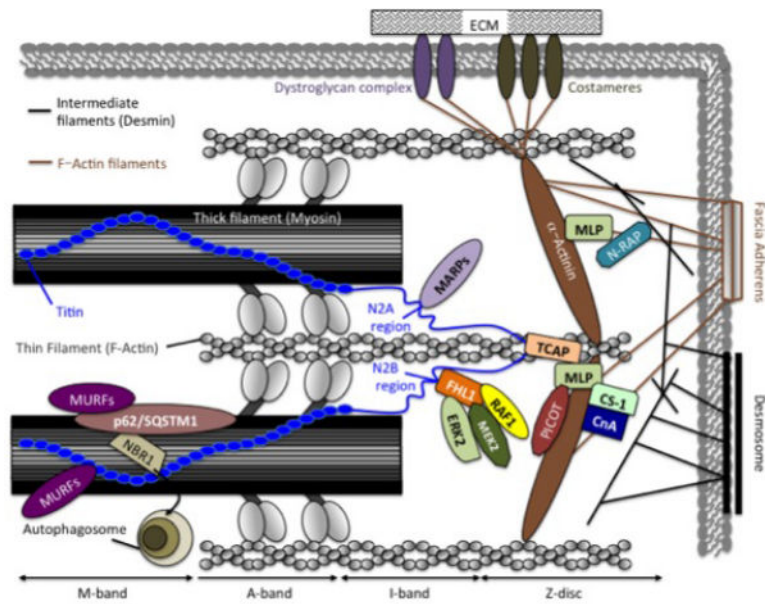


Figure 1.

A schematic representation of the specific protein complexes linked to sarcomere-mediated mechanotransduction and mechanotransmission in cardiac muscle. NBR1: Neighbor to BRCA1, p62/SQSTM1: p62/sequestosome-1, MURFs: muscle-specific RING-finger proteins, FHL1: Four-and-a-half LIM domain protein 1, ERK2: Extracellular signal-regulated kinase-2, MEK2: Mitogen-activated protein kinase kinase-2, RAF1: Rapid accelerated fibrosarcoma-1, MARPs: Muscle-specific ankyrin repeat proteins, CS-1: Calsarcin-1, CnA: Calcineurin A, TCAP: Titin Cap, MLP: Muscle-specific LIM domain protein, PICOT: Protein kinase C-interacting cousin of thioredoxin, N-RAP: Nebulin-related anchoring protein. (Illustration Credit: Ben Smith)

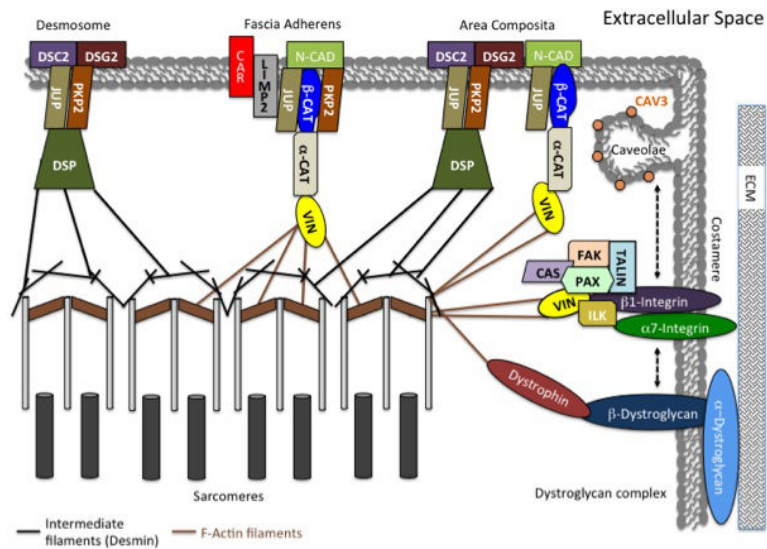


Figure 2.

A schematic representation of the specific protein complexes linked to cell-cell junction and sarcolemma-mediated mechanotransduction and mechanotransmission in cardiac muscle. Dotted arrows highlight cross-talk between integrin and caveolin as well as integrin and the dystroglycan complex. DSC2: Desmocollin-2, DSG2: Desmoglein-2, JUP: Plakoglobin, PKP2: Plakophilin-2, DSP: Desmoplakin, VIN: Vinculin, β -CAT: β -catenin, α -CAT: α -catenin, N-CAD: N-Cadherin, CAR: Cocksackievirus-associated receptor, CAV3: Caveolin-3, ILK: Integrin-linked kinase, FAK: Focal adhesion kinase, PAX: Paxilin, CAS: p130 CRK-associated substrate, ECM: Extracellular matrix. (Illustration Credit: Ben Smith).