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REVIEW

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Cardiac mechanotransduction and implications for heart disease

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Abstract Mechanotransduction, the conversion of a mechanical stimulus into a cellular response, plays a fundamental role in cell volume regulation, fertilization, gravitaxis, proprioception, and the senses of hearing, touch, and balance. Mechanotransduction also fills important functions in the myocardium, where each cycle of contraction and relaxation leads to dynamic deformations. Since the initial observation of stretch induced muscle growth, our understanding of this complex field has been steadily growing, but remains incomplete. For example, the mechanism by which myocytes sense mechanical forces is still unknown. It is also unknown which mechanism converts such a stimulus into an electrochemical signal, and how this information is transferred to the nucleus. Is there a subpopulation of mechanosensing myocytes or mechanosensing cells in the myocardium? The following article offers an overview of the fundamental processes of mechanical stretch sensing in myocytes and recent advances in our understanding of this increasingly important field. Special emphasis is placed on the unique cardiac cytoskeletal structure and related Z-disc proteins.

Keywords Z-disc proteins · Stretch response · Tensegrity · Myocardial stretch sensing · Cardiomyopathy

Abbreviations *BNC:* Brain sodium channel · *CARP:* Cardiac ankyrin repeat protein · *MLP:* Muscle LIM protein · *SAC:* Stretch-activated channel

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Introduction

Cell volume and growth regulation is essential for cell survival, and it has been postulated that mutations in mechanosensor genes cause cancer [1] and neuropathies [2]. Stretch is also known to induce muscle growth and was first reported by Csapo and coworkers [3]. Since this initial observation it has been reported that mechanical overload on remaining myocytes after both myocardial infarction and hypertension also induce a (compensatory) hypertrophy. Therefore it is conceivable that mutations in the genes responsible for mechanical stretch sensing in myocytes influence the myocardial stretch response and represent a basis for cardiac disease. In this context it is important to note that a vast number of different

mutations are known to cause cardiovascular diseases (for more detailed reviews see [4, 5], but the underlying mechanisms, leading from DNA mutations to the complex phenotype in vivo are often unknown. Studies in integrin α_7 deficient mice which develop a muscular dystrophy [6], studies in mice harboring a myocyte specific loss of integrin β_1 which develop heart failure [7], and studies in mouse models and in humans [8] have pointed to a connection between failure in stretch sensing mechanisms and heart disease.

Mechanotransduction is a highly conserved process and can be found in a wide variety of different cells, including endothelial cells, fibroblasts, and cardiomyocytes. However, the presence of a stretch sensing mechanism in these cell types does not mean that they all share a common mechanism. It is more likely that during evolution under different circumstances different sensing mechanisms were developed in different cell types and organisms. Evidence for this notion can be found in the fact that no homologues of the mechanosensitive channel in bacteria *mscL* have been found in eukaryotes, and that close homologues of the putative channel subunits in *Caenorhabditis elegans*, MEC-4, and MEC-10 have not been found in vertebrates (for review see [2]). Therefore to understand sensing mechanisms in different cell types each single cell type must be analyzed independently.

Interestingly, different forms of mechanical stretch result in the activation of different signaling pathways in a certain cell type, whereas different cells may respond to the same challenge with different biochemical answers. For example, in cardiac fibroblasts, angiotensin II activates mitogen-activated protein kinases through an upstream regulatory complex including the $G\beta\gamma$ subunit of G_i protein, tyrosine kinases including Src family tyrosine kinases, Shc, Grb2, Ras, and Raf-1 kinase, while G_q and protein kinase C are major signaling molecules activated by angiotensin II in cardiac myocytes [9].

Conversely, different forms of stretch (i.e., direct mechanical stretch or stretch induced by hypo-osmotic pressure) induce different reactions in the same cell type. For example: hypotonic swelling induced c-fos gene expression in cardiomyocytes was abolished by tyrosine kinase inhibitors, but not by inhibitors of protein kinase C, phospholipase C, or angiotensin II antagonists [10]. In contrast, c-fos gene induction by directly stretching cardiomyocytes plated on silicon membranes was inhibited by tyrosine kinase, protein kinase C, and phospholipase C inhibitors or angiotensin II antagonists [11, 12]. In addition, extracellular matrix proteins (e.g., laminin, fibronectin, vitronectin) are involved in the stretch response and activate signaling kinases in cardiac fibroblasts [13].

To date the molecular identity of a mechanosensor or mechanosensors in cardiomyocytes is unknown. Two major paradigms have emerged: a localized model of mechanotransduction in which the cellular signal is generated in close proximity to the plasma membrane and a decentralized model in which the forces applied at the cell surface are transmitted to other locations via the cytoskeleton [14, 15]. The two models are not mutually exclusive; they may exist in parallel and they may communicate.

Centralized models

Stretch-activated channels

Localized models of mechanotransduction propose that a stretch signal is generated in close proximity to the membrane; hence, stretch-activated channels (SACs) are good candidates for mechanosensors in this model. SACs, found in more than 30 different types of cells including animals, plants, fungi and even bacteria [16], are permissive to K^+, Na^+, and Ca^{2+}. Stretch of cardiac myocytes increases intracellular calcium levels [17], which can be blocked by streptomycin and gadolinium, known inhibitors of SACs [18, 19, 20]. SACs can open rapidly and amplify the signal by permitting the entry of large numbers of ions (for review see [21]), thereby demonstrating mechanosensor properties. In contrast, several other studies were unable to show that inhibitors of SAC block stretch induced expression of immediate early genes and protein synthesis [22, 23, 24].

Mechanosensation (mec) is the transduction of mechanical forces into a cellular electrochemical signal, enabling living organisms to detect touch; vibrations, such as sound; accelerations, including gravity; body movements; and changes in cellular volume and shape [2].

A genetic screen of *C. elegans* with defects in mechanosensation resulted in the identification of several mutant mec genes [25]. A subset of these genes encode cytoskeletal genes or ion channels such as degenerins. The mouse orthologue of a mec, brain sodium channel 1 (BNC1), was targeted and BNC1-null mice were analyzed. There was only a mild electrophysiological phenotype in neurons but no behavioral deficits were documented, suggesting other members of the gene family have compensated for the defect [26]. Other genetically targeted mice harboring deleted alleles of epithelial sodium channels (relatives of the BNC genes) die of defects in electrolyte metabolism within a few days of birth (reviewed in [27]).

Integrins

Integrins represent another group of transmembrane proteins, possibly involved in mechanosensing [28]. Integrins are heterodimeric transmembrane receptors that couple components of the extracellular matrix or neighboring cells with the intracellular actin cytoskeleton. Because the cytoplasmic domain physically associates with multiple cytoskeletal proteins (e.g., talin, tensin, vinculin, paxillin, and α-actinin) integrins may serve as mechanosensors, transmitting mechanical signals to the

cytoskeleton. Its interaction with the focal adhesion kinase is therefore of special importance. Focal adhesion kinase interacts with a variety of different molecules such as Src, Fyn, $p130^{Cas}$, and Graf (GTPase regulator associated with focal adhesion kinase). These signaling molecules further activate various downstream proteins such as $p21^{ras}$, mitogen-activated protein kinases, Rho/Cdc42, phosphatidyl inositol 4-5 biphosphate kinase, protein kinase C, and $p70^{S6\,K}$. Therefore integrins are also very attractive candidates for mechanotransduction.

A subset of integrins specifically binds to the amino acid sequence Arg-Gly-Asp (RGD). Pretreatment of cardiac myocytes with RGD peptides inhibited features of hypotonic cell swelling induced activation of downstream signaling [22, 29]. Because of their interaction with a variety of cytoskeletal proteins integrins might link centralized and decentralized models of mechanosensing and transduction.

Second messengers

Second messenger systems, such as nonreceptor-type tyrosine kinases (e.g., Src) have also been linked to stretch sensing. Membrane stretch might directly cause conformational changes in these molecules and activate them. Indeed, tyrosine kinase activity is observed within 5 s after hypotonic stress, the earliest time point examined following a stretch [10]. The underlying mechanism activating these kinases remains unclear. Experiments with pharmacological inhibitors suggest that protein kinase C, mitogen-activated protein kinases (extracellular signal regulated kinases 1 and 2), or even G proteins may not be involved. A vast number of additional molecules such as phospholipases C and D as well as ion exchangers such as the Na/H exchanger are implicated in mechanosensor functions (for an overview see [30]).

Given the large number of different molecules with the potential to sense mechanical force and to interact with the cytoskeleton, a reductionist approach, whereby single molecules are pointed out, might not necessarily be the strategy to solve the problem of primary mechanosensation.

Decentralized models

Decentralized models of mechanotransduction propose that mechanical stress applied at the cell surface is transmitted throughout the cell via the cytoskeleton. The term "tensegrity," based on R. Buckminster Fuller's geodesic dome, has been applied to describe the transmission of mechanical forces from one part of the cell to another. This would theoretically allow the process of mechanotransduction to occur at a locus distant from the site of applied strain [31, 32].

The tensegrity model of mechanotransduction is supported by a variety of different data, including the fact that isolated myocytes in vitro, after the application of a mechanical stimulus, are able to respond with an increase in their gene expression, protein synthesis and cell size [33, 34]. The ability to sense stretch does not necessarily depend on humoral or neural factors but on an intact stretch sensor complex inside the cell. These and other data using a variety of different agents and antagonists gave rise to the hypothesis that the whole cell is the mechanosensor [35]. However, it is likely that some molecules or macromolecular structures have more important implications in mechanotransduction than others.

Titin, T-cap, and MLP

Titin, transcribed from a single gene consisting of 363 exons and located on chromosome 2, encodes a protein consisting of up to 38,138 amino acids with a mature molecular weight of up to 4.2 MDa. It spans a half sarcomere, the contractile unit of striated muscle [36]. This gigantic protein plays a pivotal role in a variety of different processes, including myofibril assembly and maintenance. It also contributes significantly to the intrinsic passive elasticity. The aminoterminal half of the protein is anchored into the Z-disc, where it binds a variety of different proteins, including α-actinin and t-cap [37]. T-cap or telethonin, with a molecular weight of about 19 kDa is a relatively small protein with no significant homologies to any other known protein. It binds at the lateral boundaries of the Z-disc to the aminoterminal end of titin (hence its name: titin cap, t-cap). Interestingly, mutations in this protein have been found to cause a form of limb girdle muscular dystrophy [38] and it is likely to be implicated in the genesis of cardiomyopathy [8].

T-cap also binds to a variety of different proteins including the potassium channel β-subunit minK, providing at least a structural basis for the interaction of cytoskeletal proteins with ion channels [39]. Another binding partner for t-cap is the muscle LIM protein (MLP). This protein, with a molecular weight of about 26 kDa, is also relatively small. Mice deficient for MLP develop a severe form of dilated cardiomyopathy, a syndrome characterized by enlargement and impaired function of one or both ventricles.

Interestingly, the cardiomyopathy phenotype of $MLP^{-/-}$ mice can be rescued if this line is crossed into phospholamban deficient mice, generating MLP-phospholamban double-knockout animals. This can be documented by a variety of different in vivo or in vitro measurements, including electron microscopy [40]. In the case of the MLP deleted mice, a broad and distorted Z-disc can be observed, whereas this phenotype is completely rescued in case of the MLP-phospholamban double-deficient mice [8].

The underlying mechanism of the development of cardiomyopathy in $MLP^{-/-}$ seems to be at least partly a loss in the stretch sensing apparatus, represented by a macromolecular complex consisting of MLP/t-cap and

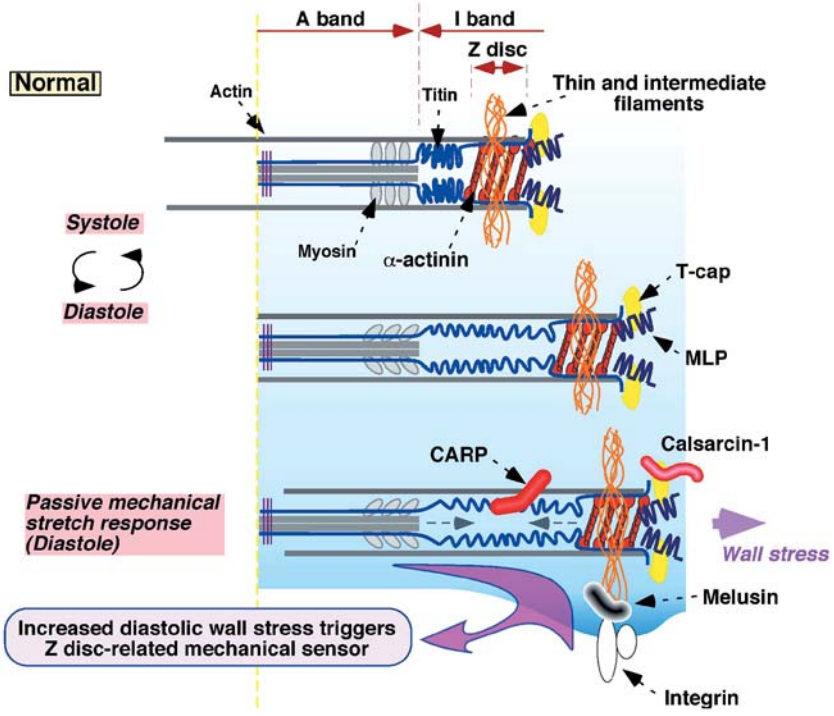

Fig. 1 Schematic diagram of a half sarcomere with different Z-disc proteins and their localization. T-cap, MLP, α-actinin, and the calsarcins are Z-disc proteins. Melusin interacts with the integrins β^1. CARP is located within the I band. Titin, a giant protein spanning a half sarcomere, is anchored with its aminoterminus at the Z-disc. During passive mechanical stretch and under physiological conditions a macromolecular complex consisting of T-cap, MLP, and titin functions as a stretch sensor (Adaptated from [8])

titin (Fig. 1). Thus the additional deletion of phospholamban in the MLP deficient background represents a strategy to overcome a defect in myocardial stretch sensing. With a more compliant passive elasticity, mechanical stretch is not sensed appropriately and hence there is failure to activate second messenger systems or to induce strain-dependent modification of the cytoskeleton itself.

The molecular basis for the higher compliance in the MLP-deficient myocardium needs to be unraveled in much more detail. Different models are conceivable: one possibility consists of a change in conformation following the loss of MLP followed by t-cap instability at the periphery of the Z-disc. Another possibility is the mechanical instability of the Z-disc, ultimately leaving titin molecules without their anchor, giving rise to a much more compliant A-band. Another possibility is a change in the expression of different titin isoforms. N2B titin, which is expressed only in myocardium, is the only isoform expressed in small rodents, representing a stiffer form of titin, whereas the N2BA isoform is much more compliant and found to be coexpressed in large mammals, including humans. Heart failure related isoform switching is at play at least in the canine and human myocardium, suggesting that this mechanism may be utilized to adjust diastolic stiffness during heart disease [41, 42]. However, whether such a change occurs in the mouse myocardium needs to be clarified.

Other possibilities include changes in the recently discovered interaction between PEVK titin and F-actin. The data suggest that this contributes significantly to passive stiffness of the sarcomere [43]. Yet another possibility includes changes in N2B titin phosphorylation by protein kinase A [44]. Phosphorylation of N2B titin might cause a destabilization of native structures within the N2B element, causing it to extend and lower its fractional extension. Considering that the activation of protein kinase A via β-adrenergic stimulation constitutes a major regulatory pathway in the heart, the protein kinase A responsive element of cardiac titins may allow modulation of diastolic function in vivo and might be at play in heart failure as well.

Other possibilities include the influence of titin on actin-myosin filament interaction and its calcium sensitivity, all of which can affect either directly or indirectly passive myocardial stiffness.

Melusin

Melusin is a muscle specific protein located at costameres near the Z-disc, where it binds to the cytoplasmic domain of β_1 integrin. Inactivation of this gene does not affect cardiac development or basal function but leads to a reduced left ventricular hypertrophy and a transition to dilated cardiomyopathy following aortic constriction. More interestingly, deficiency of this protein is not associated with any loss of sensitivity to humoral factors such as angiotensin II or phenylephrine in terms of a myocardial hypertrophic response. Further analysis revealed that following pressure overload glycogen synthase kinase 3β and Akt phosphorylation was blunted in melusin-deficient hearts [45]. Thus melusin is an element of the integrin-dependent cardiac mechanosenor and may be indispensable for the heart to induce adaptive cardiac remodeling. Several questions, such as whether human

mutations occur in this protein, and whether it might serve as a therapeutic target in heart failure, remain unanswered. The function of this very interesting molecule in different signaling pathways must be elucidated in much more detail.

Calcineurin and calsarcins

The serine-threonine phosphatase calcineurin is expressed in multiple tissues and consists of a catalytic A subunit and a regulatory B subunit. While a single gene encodes calcineurin B, three different calcineurin A subunits (CnAα, CnAβ, CnAγ) have been described in vertebrates and have largely overlapping expression patterns in various tissues. Elevations in cytoplasmic calcium concentrations promote the association of calmodulin with calcineurin and consequent activation of the enzyme. Calcineurin dephosphorylates the nuclear factor of activated T-cells transcription factor family, thereby unmasking nuclear localization signals on these proteins, which in turn results in translocation of nuclear factor of activated T-cells proteins to the nucleus and activation of transcription. This process was first documented in T-cells where it leads to the activation of immune response genes and was later found in the heart where it activates the hypertrophic response [46]. Interestingly, calcineurin interacts with calsarcins 1, 2, and 3, a recently described Z-disc associated family of proteins [47] (Table 1). By binding to the other Z-disc proteins α-actinin, γ-filamin, cypher, and t-cap [48], calsarcins provide a connection between calcium dependent signaling and the Z-disc. Hypothetically, mechanical stress can be sensed by the Z-disc and be efficiently translated into the calcineurin pathway. There is also evidence for a role of calcineurin in electrical remodeling during hypertrophy as well.

Pressure-overload hypertrophy induced by thoracic aortic banding in mice resulted in an increase in L-type Ca^{2+} channel density but no significant alteration in I_{to}. Cyclosporin A, an calcineurin inhibitor, prevented the hypertrophy and increase in Ca^{2+} current in aortic-banded mice, indicating a specific role for calcineurin in electrical remodeling [49].

Enigma/ENH/cypher family

The enigma family is a newly emerging PDZ-LIM family of proteins, defined by an aminoterminal PDZ domain and one to three carboxyterminal LIM domains [50]. Several family members are expressed in striated muscle and localize at the Z-disc, including enigma [50], actinin-associated LIM protein [51], ENH [52], and cypher [48]. Interestingly, ENH [52], enigma [52], and cypher [48] are all shown to bind to protein kinase C. A few genes in this gene family have been knocked out and the resulting animal models develop different phenotypes. For example, the actinin-associated LIM protein knockout mouse develops a pronounced right ventricular cardiomyopathy.

Table 1 Z-disc proteins and interacting partners: representatives of Z-disc proteins and their interactions with signaling molecules, ion channel-subunits, and transcriptional regulators

Protein	Interaction/properties	Reference
Cypher	Protein kinase C	48
	Calsarcin	47
ENH	Protein kinase C	52
Enigma	Protein kinase C	52
T-cap	MinK[a] (potassium channel subunit)	39
Muscle LIM protein	Nuclear protein, cofactor of transcription	57
FHL2[b]	Apoptosis induced by overexpression	58
Myopalladin	Cardiac ankyrin repeat protein	54

[a] The β-subunit of the slow-activating component of the delayed rectifier potassium current [I(Ks)] channel
[b] Four and a half LIM domain protein

In this case most probably a developmental pathway is responsible for this phenotype [51]. Cypher is expressed in at least six different isoforms and plays an essential role in striated muscle structure and function. Mice homozygous null for cypher die during the first 1–5 postnatal days because of congenital myopathy with symptoms that include decreased milk intake, limb muscle weakness, cyanosis, and cardiomyopathy [48]. Electron microscopy studies revealed severely disorganized skeletal and cardiac muscle with discontinuous/punctuate Z-discs. These findings are somewhat more severe but similar to defects found in MLP-deficient mice [8]. The fact that homozygous knockout animals die after birth implies that cypher is not necessary for myofibrillogenesis, but stabilizes Z-discs after contraction has started.

In a recent work in which two different skeletal muscle specific isoforms were knocked into the cypher locus, a partial rescue was obtained, with animals surviving up to 1 year [53]. These animal models might provide us with new tools to study skeletal and cardiac muscle specific effects of different cypher isoforms.

Additional Z-disc proteins with implications in cell signaling

Myopalladin (MW 145 kDa) and the related ubiquitously expressed protein palladin contain Ig-domains and are enriched at sites of actin filament anchorage [54]. The carboxyterminal domain is conserved between the two proteins and is responsible for its association with α-actinin. Myopalladin interacts with the SH3 domain of both nebulin and nebulette via its proline rich domain and via its N-terminal domain with the cardiac ankyrin repeat protein (CARP) within the I-band. CARP is localized both in the nucleus and along myofilaments and may be involved in gene expression [55, 56]. Overexpression of the aminoterminal myopalladin disrupts Z-disc organization and overall sarcomere structure in chick cardiac myocytes. This surprising result suggests that the inter-

action between myopalladin and CARP is a connection between myofibrillar organization and gene expression.

Summary

In conclusion, very sophisticated ideas of mechanotransduction including centralized and decentralized models have been developed. They do not mutually exclude each other, they might exist in parallel, and they may communicate with each other. Titin and the Z-disc related proteins have a variety of functions that are not limited to simply providing mechanical stability and passive stiffness. They are obviously involved in mechanotransduction, cell signaling, and gene expression. The tuning of the passive elasticity of titin is complex and involves different mechanisms with short term (protein kinase A dependent) or long-term (differential splicing, transcription) regulation. The exact pathways are not yet known but are involved in mechanotransduction. Our data on cardiac stretch sensing supports the tensegrity model of mechanotransduction, whereby specific Z-disc proteins interact with titin and are able to sense mechanical deformation. Our understanding of passive elasticity, its connection with mechanotransduction, and the resulting stretch response with implications in human disease is just beginning. Mutations in genes affecting mechanotransduction might define a new subset of cardiomyopathies with implications for therapy. It is clear that inside-outside signaling and outside-inside signaling are equally important in physiology and disease. Further work is needed to unravel the details of mechanical stress dependent signal pathways and to identify consecutive therapeutic strategies to prevent disease or stop its progression.

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