At the Crossroads of Myocardial Signaling: The Role of Z-Discs in Intracellular Signaling and Cardiac Function

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Abstract—Understanding the molecular interactions among components of cardiac Z-discs and their role in signaling has become pivotal in explaining long- and short-term regulation of cardiac function. In striated muscle, the ends of the thin filaments from opposing sarcomeres overlap and are cross-linked by an elaborate array of proteins to form a highly ordered, yet dynamic network that is the Z-disc. We review here a current picture of the function and structure of the Z-disc of mammalian cardiac myocytes. We emphasize provocative findings that advance new theories about the place of cardiac Z-discs in myocardial intra- and intercellular signaling in myocardial physiology and pathology. Relatively new approaches, especially yeast two-hybrid screens, immunoprecipitation, and pull down assays, as well as immunohistochemical analysis have significantly altered previous views of the protein content of the Z-disc. These studies have generally defined domain structure and binding partners for Z-disc proteins, but the functional significance of the binding network and of the domains in cardiac cell biology remains an unfolding story. Yet, even at the present level of understanding, perceptions of potential functions of the Z-disc proteins are expanding greatly and leading to new and exciting experimental approaches toward mechanistic understanding. The theme of the following discussion of these Z-disc proteins centers on their potential to function not only as a physical anchor for myofilament and cytoskeletal proteins, but also as a pivot for reception, transduction, and transmission of mechanical and biochemical signals. (Circ Res. 2004;94:296-305.)

Key Words: signal transduction ■ sarcomere ■ diastolic function ■ myopathies ■ hypertrophy

Z-discs, which demarcate the sarcomeres, cross-link the myofilaments into a highly ordered, 3-dimensional lattice and occupy a unique position at the interface of the sarcomere, the cytoskeleton, the sarcoplasmic reticulum (SR), and sarcolemma. In longitudinal sections in the light and electron microscope, the Z-disc appears as a ≈100-nm wide Z-line, situated at the center of the I-band (Figure 1). As also illustrated in the electron-micrograph in Figure 1, cross sections of cardiac muscle preparations reveal two structural states of the Z-disc, a predominant basket weave pattern and a small square pattern. The ability of Z-discs to alter their lattice network in conjunction with changes in actin-myosin interaction supports the hypothesized role for Z-discs as mechanosensors. The striking basket weave pattern is
which is composed of the intercalated disc and cell periphery. Whereas both populations with equal affinities and kinetics the function of actin capping protein bind the barbed ends of actin filaments. Actin capping protein is to anchor sarcomeric actin to Z-disc. The exact nature of the interaction between actin capping protein, filamentous actin, and other Z-line proteins is unknown, although interaction between α-actinin and actin capping protein has been demonstrated.

α-Actinin is a critical Z-disc protein, which cross-links sarcomeric actin and plays a role in reversing the polarity of the actins on either side of the Z-line. α-Actinin consists of homodimers arranged antiparallel with the C-terminal regions bound to adjacent parallel actin filaments within the Z-disc (Figure 1). Although α-actinin has been viewed as the major Z-disc protein in all striated muscles, it accounts for less than 20% of the total protein content of Z-discs. Moreover, as will be described, α-actinin interacts with a number of proteins, indicating that actin cross-linking and reversal of polarity requires additional proteins. Two of these proteins are titin and nebulette.

As illustrated in Figure 1, a major connection of the Z-disc with the rest of the sarcomere occurs through its interaction with the giant filamentous protein titin (connectin), which is >3 million kDa. A truncated form of titin (~700 kDa), which we discuss later together with obscurin, has also been reported to be expressed in cardiac muscle. Titin is important as a template in assembly of the thick filament by acting as a molecular ruler, and is also responsible in large part for the passive tension of cardiac myocytes above slack length and for the restoration of length below slack length. Thus, titin is a bidirectional spring. N-terminal domains of titin insert into the Z-disc where the titin molecules from opposing sarcomeres overlap. The N-terminus of titin is capped by the protein, T-cap (telethonin). T-cap is a 19-kDa protein with a unique structure and so far is restricted in expression to heart and skeletal muscle. The region of titin in the Z-disc interacts with actin through 45 amino acid domains called Z-repeats. This region of titin has also been demonstrated to bind to the N-terminal regions of α-actinin, although not all studies were able to demonstrate this binding. Alternative splicing of Z-repeats provides a mechanism for diversification of Z-disc structure. Gregorio et al reported that Z-repeats (Z1 and Z2) bind T-cap in the periphery of the Z-disc. It is of interest that the regions of T-cap that do not interact with the Z-repeats of titin are rich in basic proteins and Ser/Thr residues and are predicted to be modified by phosphorylation. In fact, it has been reported that a C-terminal kinase domain of titin phosphorylates telethonin during myofibrillogenesis. Whether the state of phosphorylation of T-cap is functionally significant in the mature Z-disc remains unknown. The C-terminus of titin attaches to the M-line of the sarcomere. In spanning the full half-sarcomere, titin makes contact with the head-neck interface of crossbridges through its interaction with myosin binding protein C (MyBP-C), and possibly thin filament actins outside the Z-disc. As discussed below, interactions of titin with MyBP-C have been proposed as an important element in length-dependent activation of the myofilaments and Starling’s law of the heart.

The passive tension of cardiac myocytes, which is a critical determinant of the operating sarcomere length and of forces determining diastolic pressure, resides largely in the stress-strain relations of the titin molecule. The molecular spring is localized in PEVK domains, in which 75% of the amino acids are proline, glutamic acid, valine, and lysine. The PEVK region, which is flanked by immunoglobulin-like (Ig) repeats, has also been demonstrated to bind to actin, by a Ca2+-dependent mechanism.
involving the Ca\(^{2+}\) binding protein S100A1, which is described in more detail later.\(^{26}\) This property appears to be specialized to the cardiac titin isoform (N2B) that is predominant in small rodent hearts and is a major component of the human heart population that includes another isoform (N2BA). Determinants of passive tension thus appear to reside not only in series attachments of titin with proteins of the Z-disc, but also in the interaction with I-band actins that run parallel to titin. Communications to and from titin and Z-disc proteins provide a sensing mechanism for the cardiac myocytes to sense strain. A role for titin in biochemical signaling cascades is also indicated by the presence of multiple phosphorylation sites, some of which are specialized in the cardiac isoform and affect passive tension.\(^{27}\) In addition, the C-terminal domain of titin has protein kinase activity.

Nebulette (107 kDa), which is the cardiac homologue of the 900-kDa skeletal muscle protein nebulin, is another relatively abundant protein associated with the Z-discs in cardiac myocytes.\(^{28-30}\) Whereas ample evidence indicates that nebulin acts as a molecular ruler for skeletal muscle thin filaments,\(^{31-33}\) the much smaller size of nebulette makes it unlikely that it plays a comparable role in cardiac muscle.\(^{28,33}\) Nebulin and nebulette share sequence similarities and contain 35 residue repeats (nebulin repeats), a linker domain, and a C-terminal Src homology 3 (SH3) domain that also binds to \(\alpha\)-actinin.\(^{29,34}\) The nebulin repeats bind actin with high affinity, but there is also evidence that nebulette motifs bind troponin T and tropomyosin.\(^{35}\) The ability of nebulette to bind both actin and \(\alpha\)-actinin raises the possibility that nebulette may anchor sarcomeric actin to \(\alpha\)-actinin.\(^{29}\) The presence of SH3 domains, which are highly conserved and composed of 50 to 70 residues that commonly link to proline-rich regions of proteins important in signal transduction, indicates a role of nebulette in signaling. Yet, this is not the case with the binding partner \(\alpha\)-actinin, which is not rich in prolines. As pointed out by Moncman and Wang,\(^{29}\) other proline-rich binding partners may play a role. It is intriguing that SH3 domains have also been implicated as important in vesicular trafficking.\(^{36}\) Although of considerable interest, whether and how the SH3 domains of nebulette participate in signal transduction or vesicular trafficking remains unknown. By targeted disruption of nebulette, Moncman and Wang\(^{30}\) have provided evidence that nebulette is important in both the structure, by aiding in myofibrillar organization and possibly as a determinant of Z-band width, and function, by determining the integrity of the thin filaments.

Obscurin (\(\approx\)800 kDa) is a recently discovered Z-disc protein that shares similarities with titin in having tandem Ig domains in an elastic region.\(^{12}\) Two of these Ig domains located in the C-terminal region are specific to obscurin and interact with the N-terminal region of titin in the Z-disc. Stretching of cardiac myocytes has been demonstrated to move epitopes localized to titin and obscurin, indicating that both filaments are extensible.\(^{12}\) Apart from its structural role in the protein network of the Z-disc, obscurin has the potential for versatile signaling properties that are described later.

Z-Discs Serve as Mediators of Signaling Cascades Regulated by Cell Strain

Increasing evidence implicates the Z-disc as a critical element in the regulation of myocardial function by changes in cell stress and strain that accompany altered hemodynamic demands.\(^{37,38}\) In basal physiological states, a balance of forces exists at the Z-disc through the action of physical links between near neighbor sarcomeres. Moreover, a network of desmin containing intermediate filaments forming part of the cytoskeleton (Figure 2) connects Z-discs of adjacent myofibrils. The position of the Z-disc is at the center if the I-band throughout the changes in cell strain that occur with contraction and relaxation in each beat of the heart.\(^{2}\) This reflects a balance of longitudinal and lateral forces acting to resist distortion along the length of neighbors within the sarcomeric assembly, and the register of sarcomeres in near neighbor myofilament bundles.

Changes in cell strain sensed by the Z-disc and associated structures occur during a beat and in the short term, with beat-to-beat changes in diastolic volume. The stretch of the myocytes that occurs with the changes in diastolic volume signals an increase in active pressure developed by the myocar-
dium according to Starling’s Law. Although Starling’s Law is a widely accepted tenet of cardiovascular biology, its underlying molecular mechanism remains elusive. There is substantial evidence that the mechanism involves a length dependence of activation of the myofilaments by Ca\(^{2+}\); as sarcomere length increases between physiological limits, maximum tension increases and so does Ca\(^{2+}\) sensitivity.\(^{39}\) Although the mechanism of the length dependence is not agreed on, one theory is that increases in sarcomere length result in decreases in interfilament spacing, which increase the probability for the reaction of crossbridges with the thin filament.\(^{39}\) This would be expected to occur in a constant volume system. Cazorla et al.,\(^{24}\) however, proposed a role for titin in length-dependent activation by a mechanism in which passive stretch of titin regulates interfilament spacing. The idea presented by Cazorla et al\(^{24}\) is that with an oblique arrangement of the extensible region of titin that joins the thin and thick filament (Figure 2), a radial force is generated that modifies interfilament spacing. Integrity in the anchoring of titin to the Z-disc is critical to this attractive hypothesis. However, a role for altered interfilament spacing in Starling’s Law has been challenged by data of Konhilas et al.,\(^{40,41}\) who could find no correlation between changes in interfilament spacing, as determined using X-ray diffraction, and differences in length-dependent activation among various muscle types. Using transgenesis to specifically replace the thin filament regulatory protein, cardiac troponin I, with slow skeletal troponin I induced a blunting of length-dependent activation that also was not correlated with altered interfilament spacing.\(^{41}\) An alteration in the length dependence of activation by a specific change in the thin filament indicates the complexity of the process. A mechanism that may not require changes in interfilament spacing has been proposed by Helmes et al\(^{25}\) at least for the case of compression at short sarcomere lengths. In this mechanism, when sarcomeres are compressed (a situation simulating the end-systolic to diastolic transition), the response of the myofilaments to Ca\(^{2+}\) is depressed. Proteolysis of titin blunted this reduction in sensitivity to Ca\(^{2+}\) and reduced the rate of restoration of sarcomere length after compression. Thus, alterations in titin isoform composition and passive stiffness, which occur in various acquired and inherited diseases, are likely to be important in modifying Starling’s Law.\(^{42}\) The role of Z-disc proteins other than titin in length dependence of activation remains unclear. Z-disc proteins considered so far form an intricate structural network. As with any complex set of interacting components, there is high likelihood that a disturbance of interactions in any one component will affect others in the network.

In the long term, sustained increases in end diastolic volume and cell stretch signal hypertrophic responses leading to cell growth and remodeling.\(^{43}\) The growth may be adaptive as during cardiac development and chronic exercise, or maladaptive leading to cardiac failure and dilation. Control of adaptive or maladaptive growth of the heart involves a combination of physical and biochemical signals that interconnect the extracellular matrix, the cell surface, sarcomeres, and the nucleus. The desmin network of the cytoskeleton, which links Z-discs to each other in neighboring myofilament bundles, to the sarcolemmal, and to the nuclear envelope,\(^{37,38,44}\) is a key component that serves as a physical link between the Z-disc and the nucleus. Extracellular mechanical stresses alter the spatial arrangement of the desmin network and impact nuclear function, including gene transcription.\(^{39,45}\)

The costamere forms a center of communication between the extracellular matrix, the sarcolemma, and the Z-disc.\(^{45}\) Costameres, which are analogues of focal adhesion complexes present in nonmuscle cells, are composed of an assembly of a growing number of proteins, including α-actinin, talin, vinculin, ankyrin, β-integrins, dystrophin, and γ-actin.\(^{45}\) The costameres are localized around the circumference of the myocytes at regular intervals and in register with the Z-disc of the peripheral myofilament bundles. Desmin, an intermediate filament, forms a major physical link between the costamere and the Z-disc of the sarcomere. Cell surface integrins, which span the sarcolemmal membrane have been identified as mechanosensors in non-muscle cells\(^{46,47}\) and have a similar role in cardiac myocytes. Cytoplasmic domains of integrins are linked to Z-discs through talin and vinculin, two cytoskeletal proteins.\(^{48}\) Extracellular domains of integrins interact with laminin of the extracellular matrix.\(^{49}\) Changes in the conformation of sarcolemmal integrins could pass through talin and vinculin to Z-disc α-actinin and actin.\(^{48}\) Likewise, dystrophin links cardiac Z-discs to the sarcolemma\(^{40}\) and may provide a route for mechanical signaling to the Z-disc. Although a detailed discussion is beyond the scope of this review, it is important that changes occurring in the extracellular matrix may influence and be influenced by signaling traffic between the costamere, peripheral Z-discs, central Z-discs, and the nucleus.\(^{45}\)

Homeostasis in the long-term regulation of the processes that determine the abundance of cardiac cellular components and the relative expression of their isoforms also depend on the integrity of the Z-disc. In pathological states, disturbance in the Z-disc protein network by inherited or acquired diseases or syndromes may have catastrophic effects. In this case, proteins of the Z-disc, which undoubtedly are important in the structural and mechanical stability of the sarcomere, serve as docking sites for transcription factors, Ca\(^{2+}\) signaling proteins, and for kinases and phosphatases that affect function and gene expression. The Z-disc also appears to serve as a way station for proteins that regulate transcription and move between the Z-disc and the nucleus. These properties demonstrate and emphasize the inseparability of the mechanical, structural, and biochemical functions of the Z-disc complex. The discussion regarding the next set of Z-disc proteins reveals this multiplex function of the Z-disc. As illustrated in Figure 2, these proteins include the following: LIM proteins (muscle LIM protein, MLP; actinin-associated LIM protein, ALP), myopotillin, myopodin, cypher (ZASP, orcale), calscarsins, CARP (cardiac restricted ankyrin repeated protein), and the Ca\(^{2+}\) binding protein, S-100.

**Z-Discs as Centers in Signaling Cascades Involving Kinases, Phosphatases, and Ca\(^{2+}\)**

Evidence of an association between cardiac Z-discs and signaling proteins such as kinases, phosphatases, and Ca\(^{2+}\) binding proteins indicates an important but poorly understood potential for Z-disc involvement in the development of myocardial hypertrophy, myopathies, and heart failure. A number of intracellular signaling chemicals are fixed to subcellular structures through a group of molecules broadly
labeled “anchoring proteins.” In addition to PKC, anchoring proteins for protein kinase A (PKA) and the phosphatase calcineurin colocalize with cardiac Z-discs. The binding of signaling molecules by anchoring proteins fixes the effector near its substrate target, thereby facilitating interaction. Furthermore, anchoring proteins confine chemical signals to specific subcellular compartments, enhancing efficiency and limiting secondary effects. In some cases anchoring proteins are able to bind several signaling molecules, sometimes simultaneously. One of these signaling molecules is protein kinase C (PKC). On activation several isoforms of protein kinase C (PKC) translocate and bind to cardiac Z-discs. Although the importance of PKC in regulating myocardial function and growth is well established, its mechanism of action, including the identity of Z-disc binding partners, is poorly understood. Csukai et al have suggested that β'-COP, a PKC-binding protein that co-localizes with cardiac Z-discs, may serve as a receptor for activated C-kinase (RACK). Actin, a significant component of Z-discs, has been shown to bind PKC under physiological conditions and may anchor activated PKC, with a preference for the ε-isozyme. Two striated muscle-restricted proteins named cypher and enigma homologue protein have also been identified as possible Z-disc anchors of activated PKC. The presence of multiple PKC anchoring proteins at cardiac Z-discs, coupled with immunolocalization studies showing PKC binding at cardiac Z-discs indicates a significant role for Z-discs in the mediation of PKC-dependent signaling.

CapZ, the actin capping protein that binds and anchors the barbed ends of the thin filaments to the Z-disc may also play an important role in PKC signaling. Disruption of the interaction between actin filaments and the actin capping protein in the heart impairs myofibrillogenesis, produces gross myofibrillar disarray, and yields nonviable offspring. The subcellular confinement of the actin capping protein to Z-discs, an important structure in PKC signaling, led us to hypothesize a role for actin capping protein in PKC signaling to the myofilaments. Using a transgenic mouse model in which the Z-disc–associated actin capping protein was downregulated in the myocardium, we found that PKC-dependent regulation of myofilament function was abolished. Moreover, the reduction in Z-disc–associated actin capping protein altered the ability of several PKC isoforms to bind cardiac myofilaments. These results provide the first evidence in support of a role for Z-disc–associated actin capping protein in the transmission of signals through the PKC pathway, and further advances the hypothesis that the localized region at the thin filament-Z-disc interface is a critical juncture in the PKC signaling cascade.

Cypher-1 and its splice variants (homologues are ZASP and oracle) are Z-disc proteins with an amino-terminal PDZ domain that interacts with α-actinin. PDZ domains, which contain the signature sequence GLGF, are universally used in all biological systems studied to date as linkers among protein networks. They are commonly found in association with SH3 domains, as is the case in the Z-disc protein network, and generally interact with the C-terminal regions of proteins in the network. Importantly, there is evidence that interaction of the PDZ domain with desmin may be dynamically modulated by protein phosphorylation. Cypher-1 also binds nonspecifically to PKC isoforms at its LIM domain. LIM is an acronym derived from three genes in which the domain was first described. It is a cysteine-rich motif defined by 50 to 60 amino acids with consensus sequence CXXCX6–2HX3CX3CX16–2CX2(C/H/D) and is also associated with zinc-binding modules. A muscle-specific variant (MLP) is a LIM-only protein consisting of two LIM domains with abundant expression in heart. α-Actinin–associated LIM protein (ALP) is another Z-disc protein with LIM and PDZ domains. ALP, is a muscle-restricted α-actinin–associated LIM domain protein that enhances actin cross-linking by α-actinin, stabilizing the sarcomere during periods of stress. The PDZ domain of ALP interacts with the spectrin-like repeats of α-actinin-2. Although abundant in skeletal muscle, expression levels of ALP are relatively low in heart. Surprisingly, ALP knockout mice do not display gross histological abnormalities in skeletal muscle, indicating either a subtle role in regulation or redundancy of function of other proteins. However, Pushmostroush et al reported that transgenic mice deficient in ALP exhibit dysmorphogenesis of the embryonic right ventricle and right ventricular dilated cardiomyopathy.

Z-disc proteins also serve as a scaffold to direct the localization of phosphatases near their substrates. Calcineurin-1 is one of the growing family of striated muscle proteins, which bind to α-actinin at the cardiac Z-disc, and also binds calcineurin, a phosphatase that dephosphorylates NFAT (nuclear factor of activated T-cell). D Dephosphorylated NFAT enters the nucleus and promotes transcription. Ca2+-calmodulin–dependent regulation of calcineurin has been implicated as a critical factor in cardiac hypertrophy. The Z-disc thus may serve as a scaffold to direct the localization of calcineurin in regulating the state of phosphorylation of NFAT. The strategic location of the Z-disc at the T-tubule-SR interface permits sensing Ca2+ on a beat-to-beat basis. It is significant that the Ca2+ binding protein S-100A1 is also localized to the Z-disc. Calcinsars also coimmunoprecipitate with γ-filamin, telethonin, and ZASP (also known as oracle and cypher), the significance of these interactions require further study. It is significant that ZASP also binds PKC.

An important developing concept is that the Z-disc serves as a way station for signaling molecules. Along these lines, Liu et al report that NFATc is localized to the Z-disc of resting skeletal muscle and translocates to the nucleus after chronic electrical stimulation. Moreover, translocation of calcineurin from the Z-disc to the nucleus has also been reported following a hypertrophic stimulus of cardiac myocytes. Other signaling proteins that redistribute in the cell also use the Z-disc as a way station in their travels to and from the nucleus. Myopodin is a 85 kDa protein, which shares homology with synaptopodin, a protein rich in prolines that are evenly distributed along the primary structure. A striking feature of myopodin is that it appears to redistribute from the Z-disc to the nucleus. In the nucleus, myopodin acts to bundle actin filaments and is thought to play a possible role in mRNA transport. Myopallidin is a 145-kDa protein that links the C-terminal Src homology 3 domain of nebulette and the EF-hand motifs of α-actinin. The region of myopallidin engaged in this linkage is within a 90-kDa C-terminal region. The N-terminal region of myopallidin interacts with CARP (cardiac ankyrin repeat protein). CARP had been reported to be a nuclear protein acting to downregulate expression of cardiac genes including troponin C, myosin light
chain 2, and atrial natriuretic factor.\textsuperscript{77,78} In studies performed by Bang et al.,\textsuperscript{79} CARP was variably localized in the sarcomplasm and the nucleus, indicating a movement of CARP between these compartments. The localization of CARP-myopallidin complex was at the center of the I-band within the Z-disc. By overexpressing this N-terminal region that contains the CARP binding domain, Bang et al.\textsuperscript{80} demonstrated a severe disruption of sarcomeric structure. They concluded that apart from a potential role as a docking station for CARP, myopallidin is important in the maintenance of the structural integrity of the sarcomere. A role for myopallidin in maintaining the assembly of the Z-disc and sarcomeric structure is also indicated by data reported by Ma and Wang,\textsuperscript{79} who demonstrated that the SH3 domain of nebulin binds with proline-rich peptides of the PEVK of titin and also to myopallidin. Whether this occurs in the case of nebullette has not been determined, but seems highly likely.

Obscurin is an excellent example of a multiplex Z-disc protein that is likely to participate in signaling cascades.\textsuperscript{12} The C-terminal region that interacts with titin also contains an IQ motif that is known to be a binding site for calmodulin.\textsuperscript{80} IQ motifs serve a variety of functions in diverse proteins that may transduce Ca\textsuperscript{2+} signals but also serve as binding sites for calmodulin independent of Ca\textsuperscript{2+}. The exact function of the IQ motif in obscurin is not clear, but its localization in the region of Ca\textsuperscript{2+} release in cardiac myocytes indicates a potential role in Ca\textsuperscript{2+} signaling. Moreover, recent evidence indicates that obscurin may make a physical link with the SR, mediated by ankyrin-1.\textsuperscript{11} On the basis of data implicating ankyrin-1 in localization of Ca\textsuperscript{2+} release channels (ryanodine receptors) in the SR, Bagnato et al.\textsuperscript{81} proposed that obscurin may serve as an element in localizing proteins of the SR as well linking the myofilaments to the SR. Ankyrin-1 has also been reported to interact with titin at the Z-disc.\textsuperscript{82} A C-terminal region of obscurin also is composed of a Rho guanine nucleotide exchange factor domain (RHO-GEF domain or DH domain) that catalyzes GTP-GDP exchange, thereby activating the Rho family of small G-proteins.\textsuperscript{83} Rho is a small G-proteins involved in a wide variety of functions including cytoskeletal structure, transcriptional control, and cell cycle control.\textsuperscript{14}

The localization of S100 at the Z-disc also indicates a role for the protein network in Ca\textsuperscript{2+} signaling. In the normal, healthy heart, S100A1 is the preferential isoform in myocardial tissue.\textsuperscript{85} Because of its subcellular localization to cardiac Z-discs, in close approximation with the myofilaments, a role for S100A1 in the regulation of myofilament function has been proposed. Most et al.\textsuperscript{86} demonstrated that exogenous S100A1 protein significantly decreases myofilament calcium sensitivity without altering maximum force development. These findings are in agreement with those of Adhikari and Wang\textsuperscript{86} who reported a reduction in skeletal muscle myofilament Ca\textsuperscript{2+} sensitivity, without a change in maximum activation. Although a link between S100 protein proteins and myofilament regulation is supported by these data, the mechanism by which S100 modulates myofilament function is unknown. Adhikari and Wang\textsuperscript{86} proposed a mechanism in which S100 binding to actin modulates actin-myosin interaction. Yamasaki et al.\textsuperscript{87} have reported an interaction between S100A1 and the PEVK segment of titin, but not actin. Although Yamasaki et al failed to detect any direct interaction between S100A1 and filamentous actin, these findings raise the possibility that the modification of actin-myosin interaction by S100A1 may be mediated through one or more intermediate proteins. Alternatively, S100 proteins may modify myofilament calcium sensitivity by inhibiting the actions of PKC. Although several studies have described the inhibitory effects of S100B on the actions of PKC,\textsuperscript{88} there are currently no reported studies investigating the relationship between the myocardial S100A1 isoform and PKC.

### Z-Discs and Mechanoelectric Feedback in Ion Channel Regulation

The flow of ions across the sarcolemma is a key determinant of myocardial function. The action potential duration defines the amount of Ca\textsuperscript{2+} entering the cell, which in turn sets the degree of myofilament activation. Matching the driving ionic flux with the mechanical demands placed on the heart may be mediated through a phenomenon termed mechanoelectric feedback.\textsuperscript{89,90} In this model, myofilament force generation is coupled to one or more ion channels, such that modifications in myofilament activation are communicated to ion channels. The resulting change in open probabilities of ion channels alters action potential duration and Ca\textsuperscript{2+} transients, ultimately affecting myofilament activation.

The subcellular mechanism through which mechanoelectric feedback is mediated is not well defined. However, recent work implicates cardiac Z-discs in this feedback loop. Furukawa\textsuperscript{91} and colleagues demonstrated a physical link between the C-terminus of the Z-line protein telethonin and the $\beta$-subunit (MinK) of the delayed rectifier K$^+$ channel ($I_{\text{K}}$). In cardiac myocytes $I_{\text{K}}$ influences action potential duration by regulating cellular polarization.\textsuperscript{91,92} The interactions between $I_{\text{K}}$, telethonin, and titin forms a complex that directly links the myofibrils with the sarcolemma, providing a circuit for mechanoelectric feedback. Mutations in $I_{\text{K}}$-encoding genes are associated with several forms of long QT syndromes and most of these mutations lie within the region encoding the telethonin-binding cytoplasmic domain.\textsuperscript{93}

Communication between the myofilaments and sarcolemmal ion channels may also be mediated through a biochemical link. The cardiac Z-disc and its associated components anchor intracellular signaling molecules, including PKC and PKA, that regulate L-type Ca\textsuperscript{2+} channels.\textsuperscript{94} Kinases and phosphatases anchored at the Z-discs are placed in close approximation with T-tubular L-type Ca\textsuperscript{2+} channels, a situation that may facilitate interaction. Although numerous studies have found that L-type Ca\textsuperscript{2+} channels are substrates of signaling proteins anchored at the Z-line, to date there are no definitive reports showing a direct relationship between Z-line signaling molecules and L-type Ca\textsuperscript{2+} channels.

### Failure of Signaling at the Z-Discs and Failure of the Heart

Cardiac myocytes function in a dynamic equilibrium, balancing extrinsic stressors with intrinsically developed force. Disruption of this balance initiates a compensatory mechanisms to reestablish homeostasis. Despite the resilience of the cardiovascular system to meet the ever-changing demands of the other organ systems, chronic hemodynamic overload exacerbates the initially compensatory changes in myocardial
function and the heart descends into a viable spiral of failure. The multiplex functions and the complexity of the Z-disc indicate that a defect in the proteins could both affect the structural integrity as well as flows and transduction of intracellular signals. Cardiac Z-discs are indirectly involved in the progression of heart failure by virtue of their association with intracellular signaling molecules, such as PKC and calcineurin. Mutations in Z-disc proteins that lead to cardiomyopathies and heart failure provide the best example of the vulnerability of homeostasis to defects in signaling through this network. Analysis of failing human hearts linked mutations in the genetic code for titin to both dilated and hypertrophic cardiomyopathy. The divergence of genetic linkage to both types of cardiomyopathy may arise from whether the coding error results in abnormal stress-strain relations of titin or alterations in intracellular signaling. Olson and colleagues identified two actin mutations that are strongly associated with dilated cardiomyopathy. Both mutations are found in the immobilized end of the actin filament that interacts with the Z-disc. These particular defects in the force-producing protein have been suggested to cause failure by virtue of altered actin function rather than a loss of function. As mentioned above, hearts of mice, deficient in ALP, which appears to stabilize the Z-disc cross-linking α-actinin, demonstrate right ventricular dilated cardiomyopathy. Zhou et al have proposed cypher as a “linker-strut” that reinforces Z-disc protein interactions. Cypher-null mutant mice also develop severe cardiomyopathy and ventricular dilation. Interestingly, the cypher deletion results in abnormal Z-disc structure in contracting muscle, but not in the noncontracting muscle of the embryonic diaphragm, suggesting that cypher is not required for normal genesis of the sarcomere, but is essential to the stabilization of Z-line structure during contraction.

Experiments reported by Knöll et al provide an explicit example of the connection between altered mechanical stability of the Z-disc and dilated cardiomyopathy. Knöll et al proposed that MLP interaction with T-Cap is a “stretch sensor” complex in mechanotransduction. The basis for this proposal is that defects in this interaction are associated with dilated cardiomyopathy and heart failure linked to an MLP mutation (W4R). Moreover, sarcomeres of MLP−/− mice demonstrated wider Z-discs and misalignment of Z-disc components. The MLP−/− mice also demonstrated altered T-cap location and release from the myofilaments to the cytosolic compartment. However, further studies are required to identify the members of this complex, the nature of their interactions, and to elucidate the mechanistic link between Z-discs, passive stretch, and cardiomyopathy. It is of interest, however, that myostatin a secreted growth factor of the TGF-β superfamily, also associates with T-Cap (Figure 1), and is upregulated in cardiomyocytes after an infarct.

Members of the S100 family of EF-hand Ca2⁺-binding proteins have also been identified as intrinsic proteins regulating the hypertrophic process in heart. In cardiac myocytes, S100 proteins associate with Z-discs, intercalated discs, and the SR. Anchoring of S100 proteins to the Z-disc may occur through the α-subunit of the actin capping protein and/or the PEVK fragment of titin. Immunohistochemical analysis of failing human hearts has shown a reduction in S100A expression and an induction of the normally undetectable S100B. Tsoporis et al hypothesized that the induction of S100B expression was a compensatory response to the overload of the failing heart. To test this idea, they forced the expression of S100B in cardiac myocytes and examined the myocardial response to hypertrophic stimuli. Expression of S100B abolished both the myocardial hypertrophy pursuant with norepinephrine treatment, as well as the induction of genetic markers of hypertrophy. These findings are in agreement with the hypothesis that the upregulated expression of S100B is a compensatory response to myocardial stress.

In view of its many interactions in the cytoskeletal network it is not surprising that the integrity of the interaction of desmin at the Z-disc is critical to cellular homeostasis. In cardiac myocytes desmin surrounds and interlinks the Z-discs, as well as forming a web connecting the myofibrils, sarcolemma, costameres, intercalated discs, sarcoplasmic reticulum, T-tubules, and nuclei. The extensive network formed by desmin filaments has been proposed to perform a variety of functions including the regulation of mitochondrial metabolism, subcellular structural organization, myofibrillogenesis, and force development. Transgenic mice with a null mutation in the desmin gene develop severe cardiomyopathy, and several studies have reported desmin-related cardiomyopathies in human populations. The myocardial failure associated with desmin abnormalities is likely the product of increased cellular fragility and impaired structural integrity with a weakened support system. By contrast, Milner et al propose a scheme of dilated cardiomyopathic development in which the impairment of force transmission by desmin and other Z-disc–associated proteins is a significant contributing factor in the resulting cell death, chamber dilation, and heart failure.

A unique pool of Z-disc dystrophin also appears essential in maintaining the structural and functional integrity of the myocardium. Coding abnormalities and null mutations of the dystrophin protein are the causative factor of muscular dystrophies, including some forms of dilated cardiomyopathy. Meng et al have found a cardiac exclusive localization of dystrophin to the Z-disc. Although the details of the interaction between dystrophin and cardiac Z-discs are unknown, several reports support the hypothesis of Meng et al that dystrophin is anchored at the Z-disc through interactions with actin. A deficiency in the Z-disc–associated dystrophin is associated with a more severe form of cardiomyopathy, as compared with the cardiac insufficiencies that develop with the loss of sarcomembranous dystrophin. The high correlation between Z-disc dystrophin loss and the cardiac derangements associated with dilated cardiomyopathy provides strong evidence of the functional significance of this unique pool of dystrophin.

Challenges Facing the Study of the Z-Disc

Although it’s an exciting time for investigation of the Z-disc, as new concepts and new Z-disc–associated proteins appear, so do new challenges. A good example is the intriguing, but poorly understood finding, that nonmuscle myosin II is present at the Z-disc. Why so many proteins with important physiological functions, such as kinases, phosphatases, and a motor protein, appear localized to the Z-disc remains a puzzle. Solving the puzzle requires high-fidelity determina-
tion of the Z-disc localization of these proteins, and their movements, substrates, and binding partners. It is also critical to understand the significance of networks of interactions among the Z-disc proteins, and with membrane proteins and lipids. Do linkages between Z-disc and the SR, T-tubule, and sarcotubular system occur in the working myocyte? If so, do the interactions simply serve to keep the membrane proteins in register during the oscillations in sarcomere length? Or is this linkage involved in mechanoelectrical or mechanochemical feedback regulation? Still another challenge is to understand the role of the Z-disc as a way station for signaling molecules involved in cell development, growth, and remodeling. To what extent are the interactions altered in cardiomyopathies, especially those linked to sarcomeric and cytoskeletal protein mutations? Approaching these questions heralds a new era of investigation that must include determination of function in what extent are the interactions altered in cardiomyopathies, Investigations of tandem repeats and terminal SH3.

Acknowledgments

We acknowledge support from NIH Grants P01 HL 62426, R37 HL 22231, and RO1 HL 64035. We are grateful to Dr Margaret A. Goldstein for providing electron micrographs.

References


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Circ Res. 2004;94:296-305
doi: 10.1161/01.RES.0000116143.74830.A9

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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