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Mechanical Stress-Induced Sarcomere Assembly for Cardiac Muscle Growth in Length and Width

Brenda Russell[1], **Matthew W. Curtis**[1], **Yevgeniya E. Koshman**[2], and **Allen M. Samarel**[2]

[1] Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL

[2] The Cardiovascular Institute, Loyola University Medical Center, Maywood, IL

Abstract

A ventricular myocyte experiences changes in length and load during every beat of the heart and has the ability to remodel cell shape to maintain cardiac performance. Specifically, myocytes elongate in response to increased diastolic strain by adding sarcomeres in series, and they thicken in response to continued systolic stress by adding filaments in parallel. Myocytes do this while still keeping the resting sarcomere length close to its optimal value at the peak of the length-tension curve. This review focuses on the little understood mechanisms by which direction of growth is matched in a physiologically appropriate direction. We propose that the direction of strain is detected by differential phosphorylation of proteins in the costamere, which then transmit signaling to the Z-disc for parallel or series addition of thin filaments regulated via the actin-capping processes. In this review, we link mechanotransduction to the molecular mechanisms for regulation of myocyte length and width.

Keywords

Focal adhesion kinase; muscle LIM protein; actin filament assembly; CapZ; protein kinase C isoforms; phosphatidylinositol 4; 5-bisphosphate; myofibrillogenesis

Introduction

A ventricular myocyte experiences changes in length and load during every beat of the heart. Contraction physically shortens the myocytes during systole, while the blood entering the cardiac chambers during diastole elongates or strains the cells. Alterations in wall stress and strain are also ultimately responsible for changes in chamber geometry that accompany pathological remodeling of the adult heart in response to hemodynamic overload. Wall stress due to pressure or volume overload, or segmental loss of functioning myocardium, is transduced into biochemical signals that increase the rate of protein synthesis, alter cell shape, and increase the transcription rate of genes normally expressed predominantly during fetal life. Although the normalization of wall stress or strain by hypertrophic growth may prove to be dispensable in maintaining cardiac performance under some circumstances [1], the underlying concepts of myocyte mechanotransduction remain critically important to our understanding of

Proofs and Correspondence to: Brenda Russell, Ph.D., Department of Physiology and Biophysics, University of Illinois at Chicago, MC 901, 835 S. Wolcott Avenue, Chicago, IL 60612, Tel: (312) 413-0407, Fax: (312) 996-1414, russell@uic.edu.

the molecular mechanisms of adaptive cardiac hypertrophy, and the progression to heart failure. There is a very large literature on other triggers and signals for muscle hypertrophy, which are beyond the scope of this review, such as humoral agonists, calcium-dependence, Ca^{2+}-calmodulin dependent protein kinases, calcineurin-NFAT, and the multiple and interlinked MAP kinase pathways, to name just a few. This review focuses on mechanical stress-induced signals, and how they may ultimately produce cardiomyocyte growth.

The regulation of myocyte growth and atrophy are primarily dependent on the interpretation of, and response to mechanical stimuli. Cyclic stresses and strains occur in normal physiology, and when these stresses and strains are maintained at new levels the myocyte responds by growth or atrophy. Specifically, cells elongate in response to increased diastolic strain by adding sarcomeres in series, and they thicken in response to continued systolic stress by adding filaments in parallel [2]. Myocytes do this while still keeping the resting sarcomere length close to its optimal value at the peak of the length-tension curve. One could thus argue that cells in pathological states that produce volume overload (such as chronic mitral regurgitation, aortocaval fistula, etc.) have adapted well to a never-ending increase in end-diastolic ventricular volume through obligatory lengthening coupled to mechanosensing of continuous strain. In contrast, myocytes undergoing concentric hypertrophy in response to unrelenting pressure overload, and that are short and thick, have adapted to conditions of prolonged stress [3]. Unfortunately, both of these new shapes eventually become maladaptive since long, thin myocytes provide little total force for ejection, while short, thick cells intrude on chamber volume itself. In this review, we link mechanotransduction to the molecular mechanisms for regulation of length and width.

Sarcomere Formation in Development

All eukaryote cells have an actin cytoskeleton that is readily remodeled to alter cell shape and enable cell motility. In evolution, cell contractility becomes specialized with smooth muscle peristalsis preceding the organized sarcomeric contraction of the heart and skeletal muscle fibers. During development, this same general scheme of contractile specialization may be recapitulated. Details of these developmental processes have been the subject of much research over many years beyond the scope herein, but there are many excellent papers and reviews [4–9]. Much of the early work on sarcomere formation was done on conventional 2D-cultured cardiac or skeletal myoblast cells having only a single layer of myofibrils readily observed by labeled antibodies. Focal adhesions form at the cell membranes to anchor the internal cytoskeletal elements to the extra cellular matrix, ECM. Attachment permits actin filaments to emanate outwards. Focal adhesions mature with time, containing vinculin, α-actinin, paxillin, and other proteins. Next, premyofibrils arise near the membrane with the appearance of short sarcomeres between Z-bodies that grow apart and have longer A-bands over time. Finally, template proteins such as titin, nebulin, and myosin binding protein C set up the structural motif to organize the sarcomere, and actin capping proteins regulate the thin filament length. Recently, enhanced optical techniques are permitting study of the developing heart in live animals that will help to clarify these important sarcomere assembly processes [10,11].

Remodeling of Length and Width in Mature Muscle Cells

Although the studies on sarcomere formation during development and in cell culture have been very useful in defining specific cytoskeletal proteins and signaling pathways important in regulation of growth, the molecular mechanisms that control actin filament insertion during new sarcomere addition in the adult myocyte remain unknown. Striated muscle cells are almost crystalline in architecture and it is difficult to see how new elements might be added in a mature fiber under the constraint of continued force production by the heart. Therefore, models of *de novo* sarcomere formation that follow the sequential assembly process of premyofibril

formation initiated at the cell membrane may not be relevant to the adult cell remodeling in response to the stresses or strains encountered in the body during adaptation to hemodynamic loads. Furthermore, sarcomere addition may proceed in either a longitudinal or transverse direction to regulate cell shape and function, which is controlled by mechanisms as yet unknown.

Clues for length remodeling processes come from adult skeletal muscle, where lengthening is thought to be accomplished mainly by the addition of new sarcomeres at the ends of the fibers near myotendon junctions [12]. Fibers do not end at flat transverse discs, but at very irregular structures with some sarcomeres seen to creep ahead of others perhaps used to elongate the cell. The analogous terminal structure in the heart cell is the intercalated disc where force is transmitted longitudinally through very strong adherens junctions. We confirmed changes during length remodeling at the end neonatal rat ventricular myocytes by N-cadherin labeling [13]. Interestingly, intercalated discs are even more irregular in cardiac hypertrophy and have a denser architecture in myopathic hearts [14].

New sarcomeres can also be added in the middle of the fiber as shown in human skeletal muscle where a Y-shaped scaffold projects inwards from the membrane to splice in new, shorter sarcomeres at the Z-disk in a desmin-labeled zone [13,15,16]. The role of a Y-scaffold for sarcomeric addition was also confirmed in cardiac myocytes during rapid lengthening in culture [13].

It is also possible that actin filaments and sarcomeres can be added internally well away from the membrane. The contractile material in striated muscle is thought by many to remodel using the Z-disc, which is a transversely oriented, lateral extension of the focal adhesion. Thus, Z-discs may act as a platform for actin filament polymerization internally in addition to the membrane location. Unfortunately this perpendicular structure is missing in conventional flat 2D cultures and its absence might explain slow progress in our understanding of myocyte width regulation. Hopefully this will be accelerated now with better 3D models in culture [17] and from the zebrafish studies of Sanger and others [9].

Mechanotransduction and signaling by the focal adhesion complex of the costameres

Stress and strain are detected by the general cell mechanosensory apparatus of the focal adhesion complex [18]. Seminal observations by Ingber and colleagues [19] suggest that mechanical deformation of integrins is the proximal step in an intracellular signaling cascade that leads to global cytoskeletal rearrangements and mechanotransduction at multiple, distant sites within the cell. One or more proteins in the sensor complex are deformed, thus triggering changes in binding partners and phosphorylation. Muscle-specific costameres act as both mechanical struts and docking sites for signaling proteins moving to and from intracellular domains.

The anisotropic geometry of the cardiac myocyte, with its longitudinal and lateral structures, may allow for distinct pathways of force recognition and transmittance [20,21]. Thus, although it is apparent the mechanosensory apparatus in myocytes does convert orthogonal strains into differential biochemical signals, these mechanisms have been difficult to study. Attempts to alter myocyte mechanotransduction experimentally *in vivo* are problematic, as myocytes not only sense mechanical load but also generate mechanical force in order to ensure the survival of the organism. This paradox has led investigators to attempt to simulate the effects of *in vivo* mechanical loading using freshly isolated myocytes in primary culture. These derivative model systems have intrinsic problems of their own, including: the presence of contaminating nonmuscle cells, the absence of an authentic 3D ECM for normal cell attachment, limited cell-

to-cell communications, abnormal excitation-contraction coupling, and lack of neuroendocrine factors. Nevertheless, *in vitro* models do have the advantage of allowing for the study of mechanical load on myocyte signal transduction and growth in the absence of other confounding variables. Most studies of load-induced myocyte hypertrophy have relied upon primary cultures of neonatal or adult ventricular myocytes grown on an elastic substratum subjected to static or cyclic stretch. Results obtained correlated well with studies performed with cultured skeletal muscle myotubes [22], isolated papillary muscles [23], and the isolated, perfused rat heart [24]. Investigators have devised a variety of clever experimental approaches to ascertain the relative contributions of passive stretch vs. active tension development [25, 26], cell orientation [27–29], and 3-dimensionality [30,31] of cultured myocytes to the process of mechanotransduction. Signaling, synchronous contraction, and efficient force are in part determined by the cell-to-cell adhesion, cell-to-matrix adhesion, and alignment [32,33]. To explore the signaling mechanisms responsible for both mechanotransduction and sarcomeric remodeling, our group developed a 3D culture system [34–37] of aligned, well-attached myocytes. Culturing neonatal rat myocytes on this 3D surface provides an environment for uniform application of mechanical strain to all cells. The system also permits cells to be suddenly stretched longitudinally or transversely to study the early regulation of myocyte length and width remodeling. Methods and results of this series of studies from our group are depicted in the cartoon (Fig 1).

In the case of length remodeling, we cultured neonatal rat myocytes on microfabricated, laminin-coated silicone surfaces. These initially have the optimal, resting sarcomere length, which is increased a sudden stress causing a 10% strain that was maintained over time; the recovery of resting sarcomere length took 4 hours, or one sarcomere per hour [31]. In these conditions, we also found that the recovery process had a requirement for new protein synthesis. Furthermore, activation of the epsilon isoform of protein kinase C (PKCε) was necessary for length recovery, as nonselective PKC inhibitors, and a replication-defective adenovirus (Adv) encoding a dominant-negative mutant of PKCε prevented the restoration of sarcomere length. To assess the importance of focal adhesion kinase (FAK) and downstream components of the focal adhesion complex, myocytes were infected with an adenovirus encoding a green-fluorescent protein (GFP)-tagged, dominant-negative inhibitor of FAK (FAK-related nonkinase, or FRNK) (Adv-GFP-FRNK). Adv-GFP-FRNK also prevented resting sarcomere length recovery, whereas a control adenovirus encoding only GFP did not. Our novel culture system provided evidence that the length remodeling processes requires new protein synthesis, as well as functional PKCε and FAK [31].

Separate directional pathways are implicated by static transverse and longitudinal loading to activate stress-induced MAP kinase in skeletal muscle [38], Rho kinase in endothelial cells [39] and FAK in neonatal cardiac myocytes [17]. Details of myocyte detection to the interval, direction, and rate of strain have been made by us using phosphorylation of FAK (Y397pFAK) and extracellular signal-regulated kinase ERK1/2 (T203/Y205 and T183/Y185 of rat ERK1 and ERK2, respectively) [17]. The interval between sudden strains in myocytes was changed to test for presence of a refractory period, as based on attenuation of FAK and ERK phosphorylation, and was found to be 5 min. Myocytes are non-responsive after 5 min of sudden 10% static strain, but recover by 15 min and show FAK nuclear translocation by 30 min. Transverse uniaxial strain applied to groove-aligned myocytes increased FAK more than longitudinally-applied strain but not ERK1/2 phosphorylation (Fig 1). Thus, cellular recognition of the vector of the driving strain could potentially be initiated by this signaling cascade for subsequent assembly of longer or wider fibers. This would elegantly match growth in a physiologically appropriate direction, but this remains to be proven correct.

Mechanotransduction and signaling by the Z-disk

The Z-disk, where the thin actin filaments insert and reverse their polarity, is another important mechanotransduction site (reviewed in [40]). Titin, the longest protein known, spans the sarcomere and is ideally situated to detect changes in sarcomere length (reviewed in [41]). Titin interacts with multiple proteins within the Z-disk, including α-actinin, the titin-binding protein telethonin (T-cap) and muscle LIM protein (MLP) [40,42–44]. MLP is a member of a large family of proteins that contains one or more double zinc finger structures (LIM domains) known to directly interact with focal adhesion proteins, including integrins, to regulate cell shape, spreading, and elongation [45–47]. Knoll and colleagues [42] proposed that defects in the MLP/T-cap/titin complex leads to defects in stretch receptor function resulting in dilated cardiomyopathy. Absence of MLP caused the displacement of T-cap in a small minority of myocytes, suggesting that the loss of MLP led to a destabilization of the anchoring of the Z disk to the proximal end of the T-cap/titin complex, thereby affecting titin's role in mechanical stretch receptor function. Mice deficient in MLP developed a progressive cardiomyopathy characterized by LV chamber dilatation, decreased ejection fraction, and premature death from congestive heart failure. Isolated papillary muscles from juvenile $MLP^{-/-}$ mice demonstrated increased passive stretch properties, suggesting an intrinsic abnormality in titin function. Importantly, neonatal myocytes isolated from $MLP^{-/-}$ mice failed to increase brain natriuretic factor mRNA levels in response to 10% biaxial passive stretch, but responded normally to Gαq coupled receptor stimulation with phenylephrine, indicating a specific defect in mechanotransduction.

Given its obvious importance in sensing and transducing strain with the titin complex in the Z-disk, we studied MLP using aligned, 3D-cultured myocytes treated with pharmacological or mechanical stimuli [43,44]. Reduced contractility decreased nuclear MLP while activity or cyclic strain increased it, suggesting that myocyte contractility may regulate MLP shuttling to and from the nucleus. Furthermore, anisotropic cyclic strain of myocytes after prior treatment with a nuclear-localizing peptide to remove nuclear MLP resulted in sarcomeres with differential MLP location. Nucleo-cytoplasmic shuttling of MLP and its myofibrillar distribution may play a further role in myocyte remodeling and hypertrophy and is required for adaptation to hypertrophic stimuli [44].

There are many other cytoplasmic, costameric and myofibrillar proteins that may translocate to the nucleus in response to mechanical strain. Perhaps this translocation results from phosphorylations mediated by signaling pathways and serves to alter transcription and gene expression [42,48–52]. Further, we note that, in addition to mechanical strain, modulation of cell signaling and gene expression is driven by extrinsic factors such as cell contacts, and integrin engagement and clustering [53]. Intrinsic responses are also important and include activation of FAK [54,55] Rho kinase [56], and most importantly, PKC [57], where the epsilon isoform is perhaps the coordinating kinase in rat cardiac myocytes. The signaling molecules then transmit signals throughout the cell reaching the Z-disc where thin filament addition is regulated.

Actin filament regulation by regulation of actin capping

The shape of the muscle is determined by regulation of how and where the thin filaments are bundled. A great deal is known about the binding dynamics of actin capping to regulate actin polymerization assembly [58–60]. Capping protein dynamics and auxiliary proteins, such as nebulin also regulate the length of the filaments itself, and the degradation and ubiquitination of myofilaments may regulate growth as well [61], but those processes are not the subject of this review. Here, we address how the orthogonal signaling pathways discussed above might be important in the direction of where to insert or remove actin filaments. One could

hypothesize, for instance, that downstream capping complexes in the Z-disk could be specifically regulated for width and lengthwise remodeling.

Our specific notion is that the signaling molecules (PKCε and PIP2) loosen the Z-disc and weaken the actin binding, thus providing an opportunity to assemble new filaments when and where needed (Fig 2). There might well be specific scaffold proteins with particular signaling in each direction (e.g. PKCε for CapZ phosphorylation and length remodeling). CapZ is the muscle actin capping protein for the barbed end of the actin filament that is composed of a heterodimer of alpha and beta subunits [60,62,63]. Capping has been observed at the Z-disc where CapZ is localized, after which it nucleates filament formation [64,65]. Structural studies have shown that CapZα and CapZβ form a mushroom-like cap, with each subunit having a C-terminal tentacle important for actin capping [65–68]. In a recent model, the CapZα tentacle binds first, followed by the CapZβ tentacle to lock the cap in place [69,70]. However, the CapZα complex might be released from the barbed end leaving the mobile CapZβ tentacle attached and able to “wobble” in place or to be removed. Studies of myofibrillogenesis using fluorescent recovery after photobleaching (FRAP) demonstrated an important role for the binding affinity between specific Z-disc constituents in sarcomere formation [71], shown diagrammatically (Fig 3). In muscle, the rates of actin filament nucleation show a half-life for a cap to remain on the filament of 28 minutes, whereas the half-life to remain uncapped is about 0.2 seconds [72], demonstrating in homeostatic conditions that spontaneous uncapping of a filament and destabilization of the cytoskeleton is rare.

The role of PKCε in myocyte elongation

How do cardiac myocytes transmit mechanical signals to induce new sarcomere addition? As mentioned, our previous studies suggest a central role for PKCε in this process. We found that adenovirally mediated overexpression of constitutively active (ca) PKCε was sufficient to induce dramatic alterations in myocyte cell shape, leading to an overall increase in cell length, length-to-width ratio, and perimeter-to-area ratio [73,74]. These changes appeared similar to the alterations in myocyte shape produced by leukemia inhibitory factor via a MEK5/ERK5 signaling pathway [75]. Further, PKCε co-localizes with FAK in myocyte focal adhesions and costameres. We subsequently found that PKCε was both necessary and sufficient to induce FAK autophosphorylation at Y397 [74,76], and found that the two kinases were localized to the same region of the myocyte. We had previously shown that FAK and the focal adhesion protein paxillin were co-localized to the cell-substratum interface in control neonatal myocytes [77]. caPKCε overexpression resulted in the formation of filapodia-like projections in many of the cells. The filapodia contained striated actin filaments traversing the entire length of the projections, with periodic, paxillin-positive attachments at the cell-substratum interface, consistent with observations from our earlier studies (see above and [73,78]). caPKCε overexpression also increased FAK staining, confirming Western blotting analysis that revealed a ~150% increase in total FAK within 48 hours after Adv-caPKCε infection [78]. Intense regions of FAK and PKCε staining were also noted in a banded pattern, consistent with the appearance of costameres, along the lengths of the elongated cell projections, strongly implicating the occurrence of sarcomerogenesis. These projections terminated in FAK- and paxillin-positive focal adhesions [73]. Thus, PKCε and FAK co-localize to focal adhesions and costameres in control and caPKCε-overexpressing cells, positioning PKCε at the crux of mechanotransduction activity.

The role of PKC in communication of mechanical signals to costameres and FAK

Specific PKC isoforms appear to coordinate a downstream signaling pathway which results in local actin filament assembly at sites of new sarcomere addition. Clerk and Sugden have shown

that Gq-coupled agonists that strongly activate PKCs induce GTP-loading of Ras, Rho and Rac in cardiomyocytes [79]. Local actin reorganization is in turn dependent on GTP-loading of one or more small G proteins of the Rho family, which is required for Rho kinase (ROCK) activation and FAK autophosphorylation at costameres [74,80,81]. Indeed, there is substantial evidence supporting a role for Rho and ROCK in myofibrillogenesis. ADP ribosylation of Rho by C3 exoenzyme caused myofibrillar disassembly in cultured chick embryonic myocytes [56]. Subsequently others showed that agonist-induced myofibrillogenesis required Rho activation [82], [83]. Rho/ROCK signaling is required for FAK activation [74,80], which we have shown is necessary for stretch-induced length remodeling and new sarcomere formation [31].

Furthermore, although considerable progress has been made in our understanding of FAK signaling, there is much to be learned about how it participates in sarcomere remodeling. As indicated above, FAK undergoes translocation to costameres in response to a variety of hypertrophic signals, leading to unfolding of its N-terminal autoinhibitory protein 4.1, ezrin, radixin, moesin (FERM) domain and binding of the C-terminal focal adhesion targeting (FAT) region to talin and paxillin (see [84] for review, and Fig 1 for a diagram of differential FAK folding in response to orthogonal strain). This unfolding activates the kinase, leading to autophosphorylation at Y397. FAK autophosphorylation then provides a docking site for Src and other SH2-domain containing molecules, and stimulates FAK tyrosine phosphorylation within the kinase domain and at C-terminal residues Y861 and Y925. These latter sites provide additional docking sites for activation of MAPKs, paxillin phosphorylation, and downstream signaling. Consequently, FAK tyrosine phosphorylation is critical to FAK-dependent mechanotransduction.

The importance of FAK to cardiac myocyte mechanotransduction was confirmed in studies of FAK knockout mice [85], and mice with targeted deletion of FAK in cardiac myocytes [86–88]. Global $FAK^{-/-}$ mice died *in utero* at E8.5 with severe abnormalities in cardiac and blood vessel formation. In contrast, cardiac tissue-specific FAK deletion produced a variable phenotype, depending upon which promoter was used to express Cre recombinase. Using nkx2.5-Cre, conditional FAK knockout mice died shortly after birth with ventricular septal defects and associated malalignment of the ventricular outflow tracts [88]. A similar phenotype was observed when MLC2a-Cre was used to inactivate FAK in embryonic cardiomyocytes [86]. The majority of these mice died *in utero* with thin ventricular walls and ventricular septal defects. However, a minority of mice survived to adulthood, but demonstrated spontaneous eccentric right ventricular hypertrophy characterized by severe RV chamber dilatation, decreased myocyte proliferation, but no evidence of increased apoptosis. In contrast, when MLC2v-Cre was used to excise the FAK gene [87], animals were born with expected Mendelian frequency, survived to adulthood without overt cardiac abnormalities, and had no overt functional defects. However, hemodynamic stress induced by transverse aortic coarctation (TAC) produced striking differences in the response of the LV myocardium to pressure overload. This was manifested by a depression of heart growth with a reduced concentric, hypertrophic response to TAC. Myocyte cross-sectional area was decreased as compared to post-TAC controls. LV end-diastolic dimension was also significantly increased; however, myocyte length was not measured. These changes were also noted in aged animals, even in the absence of TAC. Thus, these elegant studies clearly demonstrate an important role for FAK in cardiac myocyte migration during early development, as well as hypertrophy in response to pressure overload.

Although FAK tyrosine phosphorylation is critical to mechanotransduction and downstream signaling, FAK also undergoes agonist- [89–91] and stretch-induced [92] serine phosphorylation, which perhaps is required for FAK to dissociate from focal adhesion binding partners, and to exit from focal adhesions and costameres [48,49]. This time-dependent

translocation of FAK may be required for a regenerative process of local assembly/disassembly of costameres to allow for actin filament insertion at the Z-disc. Thus, the assembly/disassembly of costameres is analogous to focal adhesion turnover during cell migration of nonmuscle cells [84,93].

Regulation of actin capping and uncapping by CapZ dynamics by PKC and PIP2

The impression gained by looking at electron micrographs is that sarcomeres are permanent, crystalline structures, but this is far from true. Early studies with isotope tagging showed that the thick and filament proteins exchange over time. Furthermore, degradation of monomeric molecules is much more rapid than polymers protected in a filament, so that turnover rates are highest during atrophic disassembly [94,95]. This leads to the proposition that regulation of the dynamics of the myofibrillar proteins might underlie a mechanism for remodeling in mature muscle cells. More recent work with antibody and GFP-labeled proteins has revealed relatively rapid protein incorporation and dynamic turnover.

As noted, actin filament assembly processes may be regulated by altered capping dynamics of CapZ from the barbed end of the thin filament. Therefore, CapZ dynamics were determined during rapid growth of cardiac cells *in vitro* using adenovirus expressing GFP-tagged CapZβ1 and FRAP in cultured myocytes [96]. We found that PKC and PIP2 pathways increase uncapping when myocytes are growing, which might destabilize the existing framework and permit sarcomeric remodeling to proceed (Figs 2 and 3). Regulation of CapZ binding to actin by a PKC signaling pathway is likely. There is good evidence that the neurohormonal agonists endothelin-1 (ET-1) and phenylephrine (PE) share in common the initial activation of Gq and phospholipase Cβ (PLCβ), and have direct effects on the δ and ε isoforms of PKC [97]. The necessity of PKC activation for sarcomere remodeling was discussed above. Muscle bundles with reduced CapZ levels have increased calcium sensitivity, reduced PKCβ, and in the presence of PE and ET-1, decreased PKCε at the myofilament suggesting interplay between PKC activity levels and CapZ function [40,98]. CapZ is a factor in anchoring PKCβ to the myofilament [99]. Disassociation of CapZ from the filament could perhaps explain how PKCβ overexpression leads to cardiac hypertrophy and failure [71]. Hence, cellular processes and signaling cascades most likely play a critical role occur during the sarcomeric remodeling steps characteristic of both physiological and pathological muscle hypertrophy.

Regulation of CapZ binding to actin is also possible by the PIP2 signaling pathway. The role of CapZ in cardiac hypertrophy and remodeling may involve phosphoinositide (PI) signaling. Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2, commonly called PIP2), is mainly found in lipid bilayers where it binds and activates many ion channels [100,101]. PIP2 also regulates integrin signaling, focal adhesions, and the actin cytoskeleton through binding to partner proteins including the ezrin family of which CapZ is a member [102]. Recent crystallographic and total internal reflection fluorescence microscopy studies have predicted four lipid binding domains, two in CapZα and two in CapZβ [68,103,104]. Studies with FRAP were used to interrogate cellular remodeling and the regulatory role of PI on the dynamics of α-actinin and other actin binding proteins [71,72,105]. PI binding mutants of α-actinin-GFP fusion proteins demonstrated a dependence on PI for disassociation of α-actinin from actin stress-fibers [106]. A mode of action in which PIP2 affects cellular remodeling and hypertrophic responses through alterations in CapZ binding of actin would seem plausible. Moreover, changes in FRAP recovery witnessed by us following PE and ET-1 treatment of wild-type CapZ were significantly different when PIP2 was sequestered [96]. Other CapZ binding proteins in the Z-discs might also regulate uncapping. Myocytes contain phosphatidylinositol-4-phosphate-5-OH-kinase that localizes with α-actinin at the sarcomeric Z-disc and overlays titin T12 close to the Z-disc [107]. Regulation via PIP2 may cause changes

in cytoskeletal and sarcomeric integrity. Indeed, PKC and PIP2 may well modulate these capping and uncapping processes through potential binding sites on CapZ to regulate actin filament addition lengthwise or side-by-side differentially in response to orthogonal strain vectors.

Conclusions

Striated muscle is a highly anisotropic with specialized protein complexes at the costameres and the Z-disc. These permit differential activation with orthogonal stresses to be detected and might be linked to parallel or series addition of thin filaments. In this way, the work demanded of a muscle might trigger the signaling cascades in a manner that is specifically coupled to the direction of sarcomere assembly. Much more research is needed in models where the 3D arrangements of the protein complexes and their signaling pathways are true to native myocardial cells.

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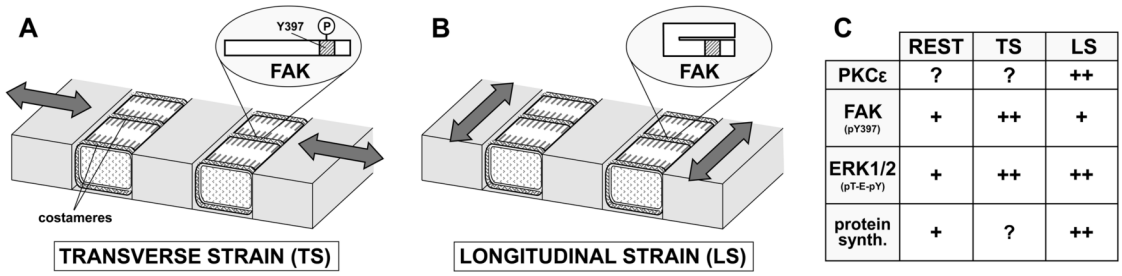

	REST	TS	LS
PKCε	?	?	++
FAK (pY397)	+	++	+
ERK1/2 (pT-E-pY)	+	++	++
protein synth.	+	?	++

Figure 1. Anisotropic mechanotransduction

Neonatal cardiac myocytes have distinct molecular responses to different directions of extrinsically-applied mechanical strain. Myocytes are aligned by culture in microgrooves and subjected to either **(A)** transverse strain (TS) or (**B**) longitudinal strain (LS) (**B**) relative to the long axis of myocytes [17]. Costameres encircle the myocyte at every Z-disc. FAK is a costameric protein whose unfolding by local mechanics may be crucial for detecting and initiating differential growth in myocyte length or width. FAK is perhaps found more often in its unfolded, active state when strained transversely than when at rest or when strained longitudinally. **(C)** The table shows major changes between spontaneously beating neonatal myocytes in this culture system compared to those with TS or LS strain. Increased phosphorylation of FAK (at Y397) was found in TS; ERK1/2 phosphorylation (at residues T203/Y205 and T183/Y185 in ERK 1 and 2, respectively) is increased in both directions. PKCε and protein synthesis are implicated in longitudinal remodeling but have not been assessed for TS [31].

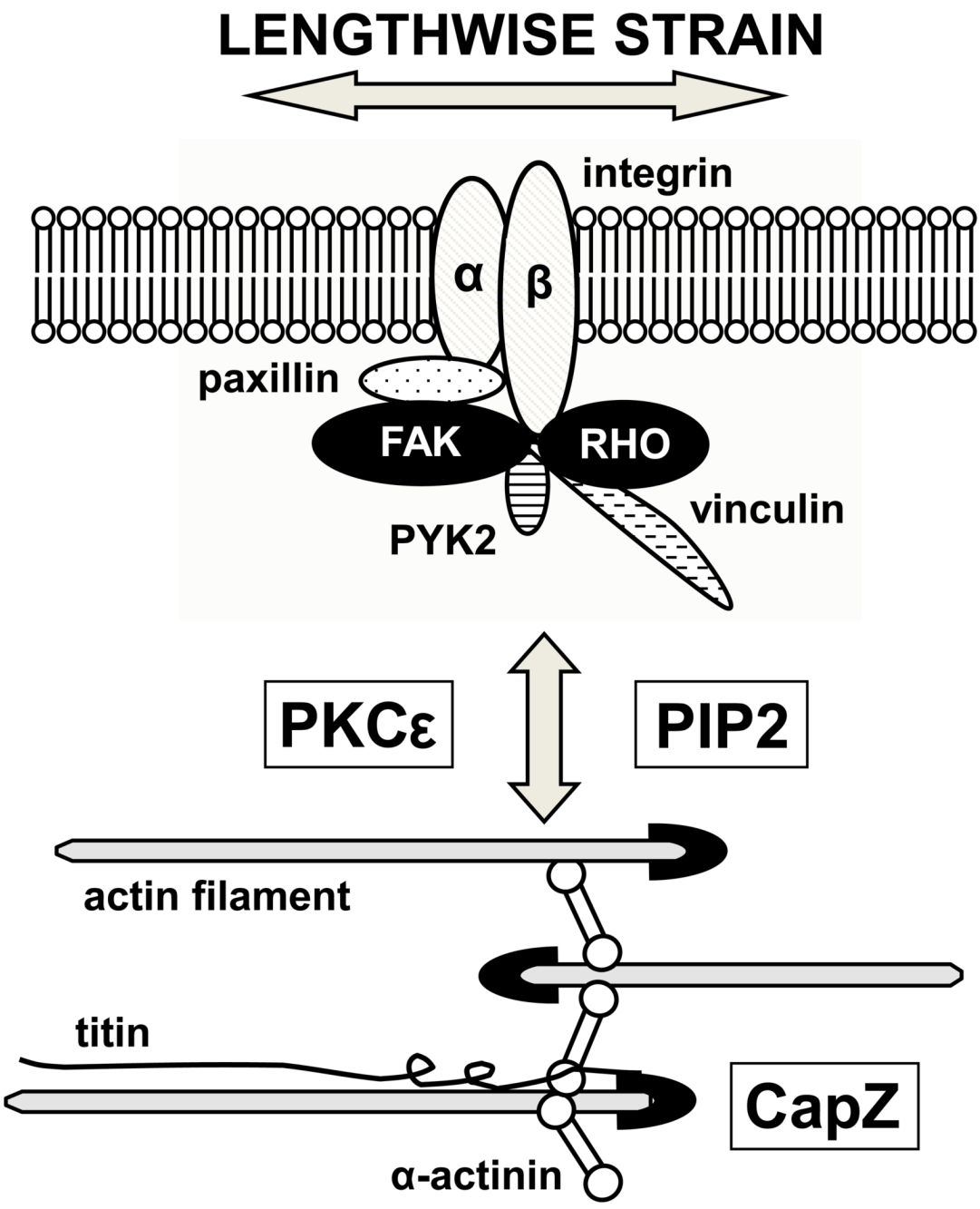

Figure 2. Lengthwise strain of mechanosensory apparatus at the costameres with hypothetical effect on filament addition at the Z-disc

Lengthwise strain is detected by the costameric focal adhesion complex at the membrane containing many proteins, shown here are integrins, paxillin, vinculin, PYK2, FAK and Rho. Longitudinal strain is detected by differential phosphorylation of some of these proteins, and then signaled via PIP2 and PKCε to the Z-disc for thin filament addition via CapZ regulation.

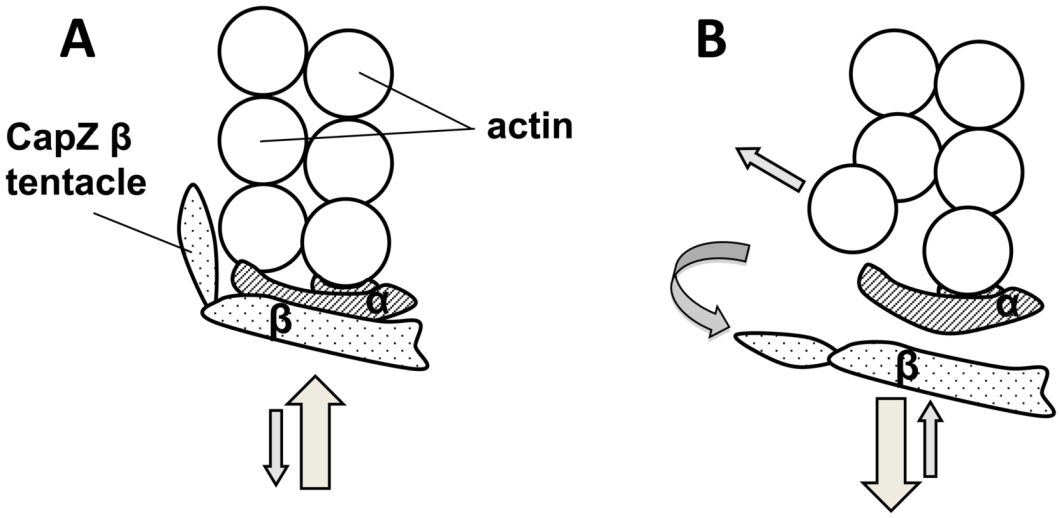

Figure 3. CapZ β-GFP binding dynamics alter with hypertrophy

(A) Actin polymerization in muscle is partly regulated by the dynamics of CapZαβ dimer binding at barbed ends of the thin filament. The α- and β-tentacles bind the CapZαβ dimer tightly to actin in normal myocytes (69, 70). (B). With hypertrophic stimuli, the CapZ αβ complex binding is more dynamic perhaps by loosening of the β-tentacle so that CapZβ-GFP has a higher off rate (96). This more dynamic state may permit greater actin remodeling and sarcomeric growth.