A broken heart: A stretch too far

An overview of mouse models with mutations in stretch-sensor components

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Abstract

With every heartbeat the heart must contract and relax. This seemingly trivial process critically needs tight control of contraction and relaxation phases, and extremely efficient coordination between these two phases to control blood flow and maintain cardiac homeostasis. To achieve this, specialized sensors are required to detect the inherent repeatedly changing environment and needs. One sensor is a stretch-sensor that monitors the filling of the ventricles. Its molecular identity and localization are only partly understood. Here we give a synopsis of the genetic models that leap into our understanding of stretch-sensors. We focus on the widely acknowledged sarcomeric sensor at the Z-disc and the costamere sensor at the sarcolemma. Recently, several novel components of both sensors were discovered. Given that these two sensors seem physically connected, it is likely that these two models are not mutually exclusive and might even communicate. We describe briefly how candidate and known proteins within these sensors receive and transduce mechanical signals in the cardiomyocyte that lead to changes in gene expression underlying homeostasis and its restoration in the heart. Emphasis is placed on the putative link between altered stretch-sensor function and heart failure observed in different genetic mouse models of stretch-sensor components.

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1. Introduction

Cardiomyocytes, specialized striated muscle cells, enable the heart to contract and relax and hence execute its function as a pump. Typically, these cells are mononucleated and self-excitable, and they contain myofibrils composed of aligned sarcomeres. These sarcomeres are multi-protein complexes that form the basic units responsible for contraction of striated myocytes, including cardiomyocytes. The sarcomeres are mainly composed of two filament systems, the thick myosin filaments and thin actin filaments (Fig. 1A). During cell contraction, these two filament systems slide past each other, much according to the ‘sliding filament theory’ that dates back

to the 1950s [1]. The sarcomeres are also responsible for the striated appearance of cardiomyocytes and can be divided in 4 major microscopically visible compartments. The Z-discs (sometimes named Z-lines) form the boundary of each sarcomere, and the region of the I-band (I for isotropic) that only contains actin-filaments and no myosin-filaments surrounds the Z-disc. The A-band (‘A’ for anisotropic) is localized between two I-bands. In the middle of this region the M-line forms the centre of the sarcomere (Fig. 1A).

The Z-disc has traditionally been viewed as a mechanical joint between two sarcomeres that is important for the cross-linking of the thin filaments and the transmission of force generated by myofilaments. Molecularly this connection is achieved by α -actinin, probably the best-known component of the Z-disc. α -Actinin serves as a bridging molecule between the distal ends of the thin actin-filaments of two

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Fig. 1. A. Schematic representation of the sarcomere. Sarcomeres are multi-protein complexes and the basic units of myofibrils. They are composed of thick (dark brown) and thin (blue) filaments. The thick filaments are composed of myosin, the thin filaments of actin. A sarcomere is demarcated by two Z-discs (brown). Titin (orange) often called the third myofilament, spans the entire sarcomere. The I-band, A-band and M-line are presented. B. Schematic representation of the stretch-sensor components at the level of the Z-disc and the costamere. The costamere comprises peripheral Z-disc molecules and subsarcolemmal proteins, and mechanically couples the sarcolemma to the sarcomere. Stoichiometric relationships are not shown and proteins are not represented to scale. Abbreviations are explained in Table 1.

sarcomeres, and therefore connects individual sarcomeres. A role for the Z-disc in cardiac homeostasis has meanwhile emerged because many signaling proteins and non bona fide structural proteins reside to this structure. Several of these signaling proteins shuttle between the Z-disc and other cellular compartments such as the nucleus, illustrating the dynamic composition of the Z-disc. Hence, it seems likely that the Z-disc can function as a highly specialized stretch-interpretating sensor [2,3].

At the cell periphery, in contact with the sarcomeric Z-disc, focal adhesion complexes exist. These structures are called costameres, and they connect the sarcomeres to the sarcolemma. They are important in transmitting contractile force generated by myofibrils to the sarcolemma and the extracellular matrix (ECM) and eventually to neighboring cells. In addition, costameres are crucial in mechanotransduction mediated mainly by integrin signaling [4].

Mutations in components of the sarcomeric Z-disc stretch-sensor and/or the costameric stretch-sensor result often in cardiomyopathies and heart failure in mice. In the first part we briefly introduce the two different stretch models, their major components and their signal transduction. These components include structural proteins but also receptors and transcription factors, illustrating the complexity of the sensors. In the following major part, the focus is on description of defects in the genetic mouse models and how these leap in our understanding of the mechanical stretch sensors and their signal transduction.

2. The stretch-sensor

Stretch stands for muscle lengthening. In the heart this occurs after active contraction, when the opposing forces override the tension that results from the contraction. The muscle is then stretched against the resistance force in a process called eccentric contraction, whereas muscle shortening is called concentric contraction. Myocytes have passive spring-like properties that help the stretched cells to return to their resting length. Intracellular proteins such as titin mainly accomplish this, but the extracellular matrix is also important in this process.

Ca^{2+} switches on muscle contraction, and reuptake of Ca^{2+} in the sarcoplasmic reticulum by the SERCA pump, relaxes the muscle. The natural inhibitor of the SERCA pump is phospholamban whose action can be reversed by β -adrenergic stimulation. Hence, chemical stress by isoproterenol (a β -adrenergic stimulus) enhances the relaxation phase. As a result the inflowing blood passively stretches the chambers of the heart.

Stretch/strain sensing and mechano-transduction are complex combined processes in cardiomyocytes. At present it is conceived that stretch signals are not sensed by a sole stretch-sensor but probably involves multiple protein complexes responsible for sensing – and transducing – stretch-linked biomechanical signals. Based on the localization of these complexes, we discuss two major models of stretch-sensors, one at the level of the sarcomeric Z-disc and another at the level

of the sarcolemma [2]. Fig. 1B gives a schematic representation of these models and can be used as a lead in the next sections.

2.1. The sarcomeric Z-disc stretch-sensor

α -Actinin anchors several crucial components of the Z-disc sensor. We first introduce the titin-Tcap-MLP complex and its interacting proteins and then highlight other Z-disc specific components.

Titin, a giant modular protein (3–4 mDa), has in its N-terminal region at least four binding sites for α -actinin. Two titin molecules span the entire sarcomere from Z-disc to Z-disc with their C-terminal portions overlapping in the M-line. Titin is, like nebulin/nebulette believed to be essential for correct sarcomere assembly by virtue of its proposed function as molecular ruler [5–8]. Probably, titin is also implicated in signaling since it harbors a Ser/Thr kinase domain in its C-terminal segment and multiple phosphorylation sites. Importantly, titin acts as a molecular spring, i.e. modulates the intrinsic elastic properties of cardiomyocytes. The region of titin that localizes in the I-band of the sarcomere contains 3 elements that provide titin with elastic properties: tandem immunoglobulin-like (Ig)-repeats, a PEVK (rich in proline, glutamate, valine and lysine) domain and a N2B element. The N2B element (composed of 3 Ig-repeats and a 572-residue unique sequence) is unique to cardiomyocytes; whereas the N2A element is present in skeletal and cardiac muscle. These 3 elements indeed form a bidirectional spring that generates force opposite to stretch or contraction thereby enabling the muscle to passively return to its original resting length. Some proteins like Tcap, MLP, calsarcin-1 and calcineurin can respond in the Z-disc to this passive titin-generated tension and belong to the emerging structure of the stretch-sensor [6].

The N-terminal Ig-repeats Z1 and Z2 of titin can bind to Tcap (telethonin) in the periphery of the Z-disc. Tcap is a 19 kDa muscle specific protein identified as a titin capping protein at the Z-disc. It has no significant homology to other proteins. It is thought that Tcap functions as a bolt to anchor titin at its N-terminus [9,10]. Overexpression of Tcap *in vitro* in cardiomyocytes disturbs Z-disc morphology, thereby showing its importance for maintenance of sarcomeric stability [11]. The close interaction between titin and Tcap, which contributes to sarcomere integrity, is further secured by muscle-specific LIM protein (MLP). MLP is an adaptor that belongs to a family of cystein-rich proteins, it is fairly small (~21 kDa), and directly interacts with Tcap. It also interacts independently with α -actinin and consequently locks the titin-Tcap-MLP complex to the Z-disc [12,13].

Another Tcap partner is calsarcin-1. Calsarcins are muscle specific proteins of the Z-disc, and only calsarcin-1 is synthesized in the heart. Interaction with Tcap occurs via the N-terminal segment of calsarcin-1 [14–16]. Tcap probably establishes via calcineurin an important link between mechanical stretch signals and local Ca^{2+} concentrations. In the absence of calsarcin-1 the Ca^{2+} -dependent phosphatase

calcineurin is activated, illustrating the inhibitory role of calsarcin-1 on calcineurin.

Remarkably, bone morphogenetic protein 10 (BMP10) was identified recently as a new interactor of Tcap. This secreted ligand of the TGF β superfamily can act as an adaptive pro-hypertrophic factor in conditions of elevated wall stress. Its action mechanism at the Z-disc is still unclear, but the colocalization of BMP10 with T-cap suggests that BMP10 is sequestered at the sarcomeric Z-disc in a complex with Tcap and may be released in the extracellular environment when increased stretch is sensed [17].

Similar to titin, actin filaments are also capped within the Z-disc. The actin capping protein capZ is a heterodimeric protein composed of an α - and a β -subunit. Myocytes contain two β -isoforms that are distributed differently in the cell, with β_1 -subunits at the Z-line and β_2 -subunits at the intercalated disc and at the cell periphery [18,19]. CapZ stabilizes actin-filaments but binds also to α -actinin-2, thereby anchoring the thin filaments to the Z-disc [20].

Other α -actinin-2-interacting proteins are PDZ and LIM domain containing proteins like cypher, α -actinin associated LIM protein (ALP), enigma, ENH and CLP36 [21–23]. The LIM domains may establish a signal transduction path through their binding to protein kinases, which is potentially also necessary for stretch sensing [24]. For example, different PKC isoforms interact with cypher, enigma and ENH [25], and in cardiomyocytes PKCs predominantly regulate contractility and hypertrophy [26].

Myotilin, S100A1 and myomaxin were recently identified as new proteins residing at the Z-disc but further investigation is required to link them to stretch sensing. S100A1 is a Ca²⁺ binding protein of the EF-hand type that regulates cardiac tissue contractility [27]. Myotilin interacts with typical Z-disc proteins like α -actinin-2 and calsarcin, but also with γ -filamin that secures sarcolemma association [28,29]. Myomaxin is a novel α -actinin-2-interacting protein related to the actin-binding protein Xin and functions downstream of MEF2A at the Z-disc [30].

2.2. The costameric stretch-sensor

Costameres are in contact with the outermost Z-discs of the cardiomyocyte and surround the cell somewhat like the metal rings around a wooden barrel. Costameres are highly specialized protein complexes in striated myocytes that function in cell-matrix attachment and bidirectional communication between the inside and outside of the myocyte [4,31]. They transmit external load to the cell's interior: when cells cultured on stretch membranes undergo a 10% stretch, this immediately results in a 10% increase in sarcomeric length [32]. Costameres can also transmit internally generated force to the environment. Flexible silicon membranes seeded with rat cardiomyocytes display pleat-like wrinkles when the cells contract. Strikingly, these wrinkles form exactly between the Z-discs of the cells, likely by firm attachment of the cells to the membrane via costameres [33].

Vinculin is a cytoskeletal protein that is found in the costamere. It is required to transmit stretch signals from the sarcolemma to actin/ α -actinin in the sarcomere [34]. Such a link between the sarcolemma and the contractile apparatus in myocytes is important for normal myocardial function and integrity [35].

Integrins are transmembrane proteins that connect components of the ECM with the actin cytoskeleton. Hence, integrins are indispensable for the bidirectional transmission of mechanical forces from the cardiomyocyte across the sarcolemma to the ECM and vice versa. In cardiomyocytes integrins are highly enriched in costameres [36]. To date, 18 α - and 8 β -integrin-subunits have been identified that can form 24 distinct $\alpha\beta$ -heterodimeric integrins. In addition, the different subunits exist in multiple isoforms as a consequence of alternative splicing. In cardiomyocytes, the α_1 -, α_3 -, α_5 -, α_6 -, α_7 -, α_9 - and α_{10} -subunits are predominantly expressed in combination with the β_{1A} - and β_{1D} -subunits [37]. Integrins interact with structural adaptor proteins such as filamin, α -actinin, tensin and talin [38], and with signaling proteins, among others focal adhesion kinase (FAK), melusin, integrin-linked kinase (ILK) and small GTPases. Most of these signals converge on AKT, RAS and ERK1/2 that can activate specific target genes. In general there is significant cross-signaling with polypeptide growth factor cascades, suggesting that various cellular responses, like cell survival and growth, are in fact locally coordinated in costameres [39].

FAK is a cytoplasmic Tyr kinase identified as a substrate for Src that localizes to cell adhesion sites in various cell types, including in cardiomyocytes. FAK plays a major role in integrin-mediated signal transduction. Clustering of integrins recruits FAK to costameres where it interacts with the integrin β -subunit but also with talin and paxillin. This leads to activation of FAK by autophosphorylation of Tyr-397.

Several studies show that FAK is important in transducing signals that lead to the development of cardiomyocyte hypertrophy. The kinase becomes activated in hemodynamically overloaded myocardium from mouse and rat and also in isolated cardiomyocytes subjected to pulsatile mechanical stretch [40–42]. Increase in left ventricular pressure is also associated with increased overall Tyr phosphorylation of FAK [43]. In isolated rat cardiomyocytes, activation of FAK is induced mainly by mechanical signals [44]. This is further supported by the fact that increased contractile activity induced by rising Ca²⁺ levels does not result in phosphorylation of FAK [43].

Melusin is, like FAK, a signaling protein implicated in stretch sensing. It is a muscle specific protein located at costameres near the Z-disc, where it binds to the intracytoplasmic tail of β_1 -integrin [45]. Studies in the mouse (see further) have shown that melusin is only involved in mechanical stretch sensing and not in chemically induced stretch [46].

ILK is an integrin linked Ser/Thr protein kinase that directly interacts via its C-terminal segment with β_1 -integrin [47]. ILK is emerging as a crucial player in mechanotransduction mediated by integrins [48]. Cardiomyocyte specific

ILK deficient mice develop spontaneous cardiomyopathy and heart failure by 6 weeks of age [49]; see further).

PARVB is a 42 kDa focal adhesion protein that interacts with α -actinin and ILK, and that inhibits the activity of ILK [50]. Recently, myospryn (413 kDa) was identified as another component of the costamere that interacts with α -actinin. Myospryn is a direct downstream target gene of the MADS box type transcription factor MEF2A [51]. Likewise, Four-and-a-half LIM domain proteins (FHLs) are LIM domain proteins that interact specifically with $\alpha_7\beta_1$ integrin, postulating a role for these proteins in stabilizing striated myocytes during mechanical stress [52].

3. How is mechanical stretch transduced?

Stretch regulation is highly conserved between cardiac and skeletal muscle. When increased stretch is sensed at the sensor this information has to be transmitted to the nucleus where gene transcription must be adjusted. In particular, a hypertrophic gene program that leads to an overall increase in sarcomeres has to be activated and is accompanied by increased expression of fetal cardiac genes [53]. Often, the counteracting gene programs of cell survival and apoptosis are also initiated [2,3,54].

In skeletal muscle MLP can translocate to the nucleus and interacts there with the bHLH transcription factor MyoD to enhance its DNA binding and thereby modulate its transcriptional activity [55]. In mice submitted to pulmonary arterial hypertension, redistribution of MLP to the nucleus is observed [56]. Also *in vitro*, when neonatal rat cardiomyocytes are stretched on silicon membranes for 48 h, this relocation of MLP to the nucleus is detected. Nuclear MLP invariably seems to be monomeric whereas cytoplasmic MLP has been found as dimers, trimers and tetramers. In aortic banded and myocardial infarcted rat hearts an accumulation of the nuclear monomeric form with concomitant decrease of the cytoplasmic oligomeric forms was reported [57]. This further confirms that increased wall pressure and stretch induce relocation of MLP to the nucleus.

A list of other candidate messengers that transmit stretch signals from the Z-disc to the nucleus is emerging. Calcineurin is a Ser/Thr protein phosphatase consisting of a catalytic A-subunit and a regulatory B-subunit. Calcineurin A has three isoforms (α , β and γ) of which calcineurin A α and - β are expressed ubiquitously, but only the β -isoform is induced upon stress in the heart [58,59]. When the cytoplasmic Ca^{2+} concentration increases, the interaction between calmodulin and calcineurin is promoted, leading to activation of calcineurin. Calcineurin dephosphorylates transcription factors of the nuclear factor of activated T-cells (NFAT) family. This leads to unmasking of a nuclear localization signal and subsequent translocation to the nucleus, which was first documented in T-cells [60] and later also shown to be crucial in the development of cardiac hypertrophy [61]. In basal conditions calcineurin and NFAT localize both to the Z-disc [62,63]. After hypertrophy-

inducing isoproterenol or chronic electrical stimulation, calcineurin and NFAT both translocate to the nucleus [64].

The 95 kDa actin binding protein myopodin bundles actin filaments in the cytoplasm and in the nucleus. Myopodin localizes along cytoplasmic actin filaments in a punctuated pattern and at the Z-disc of the sarcomere, where it co-localizes with α -actinin. Its sequence shares homology with synaptopodin and it is probably involved in the regulation and organization of Z-discs, and in differentiation of cardiomyocytes. In undifferentiated myoblasts, myopodin resides in the nucleus, but when differentiation to mature myocytes proceeds it becomes incorporated in the Z-discs. In differentiated myocytes, hardly any nuclear localization of myopodin can be detected, but after stress (heat shock) myopodin translocates again to the nucleus [65]. Myopodin likely plays a dual role as a structural protein at the Z-disc and regulatory protein participating in a signaling pathway between the nucleus and the Z-disc during development as well as in situations of cellular stress.

Another protein that can shuttle between the cardiac Z-disc and the nucleus is cardiac ankyrin repeat protein (CARP). CARP has structural and functional similarities to Ankrd2, which is specific to striated muscle. CARP and Ankrd2 belong to a family of muscle ankyrin repeat proteins (MARPs) that form a complex with titin, (myo)palladin and calpain protease p94, and that are involved in signaling and regulation of gene expression in response to muscle stress.

CARP interacts with myopalladin, the cardiomyocyte specific homolog of palladin. Myopalladin is anchored to the Z-disc by α -actinin and nebulin, and the interaction with CARP occurs through its N-terminal domain [66]. Overexpression of this N-terminal domain of myopalladin in chick cardiac myocytes severely disrupted all studied sarcomeric components, indicating that the myopalladin-CARP complex in the central I-band may regulate sarcomeric integrity [66]. CARP is an important regulator of cardiogenesis through expression of cardiomyocyte genes like troponin C, myosin light chain 2 and atrial natriuretic factor (ANF) [67].

Zyxin, a member of the LIM protein family, is also a candidate messenger to mediate signals between the cytoplasmic sarcomeres and the nucleus [68,69]. In the cytoplasm zyxin localizes to the Z-disc (via interaction with α -actinin and MLP [12,70]) or the costameres. cGMP-dependent nuclear accumulation of zyxin was observed in cardiomyocytes after ANF receptor stimulation. Subsequently, nuclear activation of AKT leads to better resistance to cell death, suggesting an anti-apoptotic role for zyxin [71].

4. Impaired stretch-sensors result in heart failure

When cardiac homeostasis is disrupted, compensatory mechanisms are initiated and operate to re-establish the dynamic equilibrium in the cardiomyocyte between extrinsic stress and intrinsically developed forces. When hemodynamic overload becomes chronic, this may exacerbate the

Table 1

Overview of components of the sarcomeric Z-disc and costameric stretch-sensor and associated signaling molecules

	Name	Synonym	In vivo mouse models
α -actinin-2	ACTN2	n.a.
actin		KO >50% $\dagger$ prenatal; remainder $\dagger$ within 2 weeks postnatal	[106]
ALP	PDLIM3	KO 15% $\dagger$ prenatal: embryonic form of DCM RV; 85% survives: DCM RV (6 mo)	[89]
			KO No cardiac phenotype	[88]
BMP10		KO $\dagger$ At E10.5: impaired cardiac growth and chamber maturation	[107]
			cOE Viable: reduced heart size, impaired postnatal hypertrophic growth	[108]
calcineurin	PPP3ca, CN, PP2B, Caln, Calna, CnA α	cOE Viable: hypertrophy (CnA); cOE CnA inhibitors: inhibition of hypertrophy	[61,82]
		CnA β	KO No cardiac phenotype	[84]
			KO Reduced heart size and reduced hypertrophic response	[85]
calsarcin-1	myozenin	KO No cardiac phenotype, but exaggerated hypertrophy upon CnA activation	[83]
capZ	Cappa, CAZ, Capza, CP	TG $\dagger$ Within 4 wks postnatal: disrupted sarcomeric organization, hypertrophy (overexpression β 2)	[19]
			TG Viable: altered morphology intercalated discs (overexpression β 1)	[19]
			TG Viable: increased Ca ²⁺ sensitivity and control of maximum isometric tension, PKC β (II) anchorage at myofilament	[86,87]
CARP	ankrd1	n.a.
CLP36	PDLIM1, Elfin, CLIM1	n.a.
cypher	oracle, ZASP	KO E17.5: disorganized and fragmented Z-discs; majority $\dagger$ P1	[90]
			GT 50% $\dagger$ within 4 wks postnatal; remainder survives to adulthood: fibrosis in cardiac muscle	[109]
ENH	PDLIM5	n.a.
enigma	PDLIM7, LMP1	n.a.
FAK	PTK2	KO $\dagger$ At E9.5: abnormal heart and impaired vessel development	[110]
			cKO Viable: eccentric hypertrophy and fibrosis after AngII stimulation, LV dilation (9 mo), disorganized myofibrils (MLC2v-Cre)	[95]
FHL2	SLIM3, DRAL	KO Viable: normal in baseline conditions, exaggerated hypertrophy upon isoproterenol induction	[111]
			KO No cardiac phenotype	[112]
γ -filamin	FLNC, FLN2, ABPA, ABPL	KO $\dagger$ At birth; heart morphology is similar to wild type	[98]
ILK	p59	KO $\dagger$ At implantation: failure of epiblast polarization and cavitation	[93]
			cKO $\dagger$ At 6–12 wks postnatal: cardiomyopathy (MCK-Cre)	[49]
			cOE Compensatory hypertrophy	[48]
β 1-integrin	fibronectin receptor- β	KO $\dagger$ At implantation	[92]
			cKO Viable: DCM after pressure overload (MLC2v-Cre)	[37]
MAFbx	atrogen1, FBXO32	KO Viable and fertile: skeletal muscle atrophy	[100]
				[46]
melusin	ITGB1BP2	KO Viable: normal baseline conditions; contractile dysfunction and DCM upon pressure overload
MLP	CSRP3, CLP	KO 50 to 70% fatigued (P5–P10) and $\dagger$ 20–30 h later; survivors DCM at adulthood	[79,80]
MuRF3	RNF30	KO Viable: hypertrophy, increased cardiac apoptosis, abnormal cardiac muscle contractility	[101]
myomaxin	CMYA3, Xirp2	n.a.
myopalladin	Mypn	n.a.
myopodin	synpo2	n.a.
myospryn	CMYA5	n.a.
myotilin	MYOT, TTID	KO No cardiac phenotype	[113]
nebullette	NEBL	n.a.	

Table 1 (continued)

	Name	Synonym	In vivo mouse models
PARVB		n.a.
paxillin	PXN	KO	† At E9.5: abnormal heart development [114]
PKCε	PKCE, PRKCE	KO	No cardiac phenotype [115,116]
S100A1		KO	Viable: normal baseline conditions, reduced contraction and relaxation rate upon β-adrenergic stimulation [117]
talin1	TLN	KO	† At gastrulation [94]
Tcap	telethonin	n.a.
titin	connectin, TTN	CI	† At E11.5: heart failure [75]
			KO	† Around midgestation: pericardial hemorrhage, reduced ventricular wall thickness and trabeculation [76]
			cKO	† Around midgestation: disassembled sarcomeres, no increase in cardiac hypertrophy related genes (αMHC-Cre) [77]
			cKO	† At 5 wks postnatal: reduced heart size, disarrayed sarcomeres (MCK-Cre) [77]
			SM	† Around 2 mo: acute and chronic myositis, no cardiac defects (mdm mutation) [74]
			KO	Viable and fertile; mutant hearts smaller (N2B-KO) [78]
vinculin	VCL	KO	† At E10.5: abnormal cardiac development [96]
zyxin	ZYX	KO	No cardiac phenotype [118]

Abbreviations: CI: chemically induced; cKO: conditional knock-out; cOE: conditional overexpression; DCM: dilated cardiomyopathy; E: embryonic day; GT: genetrap; HCM: hypertrophic cardiomyopathy; KO: knock-out; P: postnatal day; SM: spontaneous mutation; TG: transgene.

initial compensatory effort and develop into progressive heart failure. Defects in stretch sensing and subsequent force transmission are associated with cardiomyopathies and/or muscular dystrophies. Indeed, abnormalities in sarcomeric Z-disc and costameric proteins have been recognized for many years to lead to heart failure. These abnormalities may affect mechano-transduction as well as structural integrity. Here we focus on *in vivo* studies in genetic mouse models (overviewed in Table 1). Where appropriate we will briefly discuss the relation with human cardiomyopathies.

4.1. Mutants of Z-disc components

Given the crucial role of titin in sarcomeric function, it is not surprising that mutations in the titin gene cause familial dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) [72,73] and also muscular dystrophy (Limb-Girdle Type 2J) [74]. The divergence in phenotype between DCM and HCM reflects on whether the mutant titin leads to altered stretch-strain, intracellular signaling or purely structural modifications. Several titin gene mutations are known in mice, ranging from complete disruption of the protein to more subtle changes. In an ENU screen for recessive mutations that lead to developmental defects in the mouse, a mutation in the titin gene was induced that caused embryonic lethality around mid-gestation. The hearts appeared normal at embryonic day (E) 8.25, but from E9.5 onwards, severe defects such as pericardial edema and weak contractility were observed. However, cardiomyocytes did

not show DCM features, but the cells were cuboidal and misaligned. These defects are probably the result of impaired myofibril assembly [75]. Germline and conditional knockout mice with a deletion of the M-line segment of titin have been generated. They all have a different phenotype depending on the knockout strategy used. When the M-line region of titin is deleted in the germline, mice die during embryonic development due to heart failure. Also a conditional M-line segment knockout generated with the αMHC-Cre mouse results in embryonic lethality around mid-gestation. In these mutant embryos, the cardiac sarcomeres are disassembled from E10.5 onwards, but no increase in hypertrophic response-related gene expression is observed [76]. Using the MCK-Cre mouse for the generation of an M-line segment deficient titin mutant results in a milder phenotype, but also these mutants suffer from cardiac failure and die around the fifth week after birth. The two different promoters used to drive Cre expression can most likely explain the different phenotype in these two conditional M-line segment knockout approaches. The αMHC-promoter becomes active earlier in development than the MCK-promoter, resulting in an earlier ablation of the M-line segment of titin and consequently a more severe phenotype. A common feature in all these titin M-line mutants is the disarray of sarcomeres [76,77]. Furthermore, a defective interaction of titin with Tcap is observed and the mice show reduced heart sizes [77]. In a titin mutant where the N2B domain was deleted, interaction with FHL2 and alpha-B crystalline was impaired but these survived to adulthood [78].

Tcap interacts with titin, MLP and calsarcin-1 (see above, see also Fig. 1). The clinical relevance of these interactions is illustrated by mutations in these genes in a subset of patients with DCM and HCM [15,79]. Knoll and co-workers [79] have shown that a DCM associated mutation in the MLP gene displays a defect in its interaction with Tcap. In general, DCM associated mutations result in impaired interaction of Tcap with its partners, which makes the cardiomyocytes more prone for extension due to reduced passive tension. In contrast, HCM associated mutations result in increased interactions of Tcap with titin, MLP and calsarcin-1, leading to increased passive tension.

Mice with deficiencies in MLP are reported to exhibit chamber dilation and contractile dysfunction characteristic for DCM. This phenotype is accompanied by cardiac hypertrophy, interstitial fibrosis and heart failure. The cardiac abnormalities are not obvious yet at birth, but they develop soon after [80]. However, ultra-structural analysis of MLP-deficient hearts shows that disruption of the cardiomyocyte architecture with concomitant myofibrillar disarray is already present perinatally. Thus, disorganization of the cyto-architecture precedes the onset of DCM, which is rather unusual, but indicates that MLP is necessary for normal myofibrillar organization. Dependent on the genetic background the outcome of the MLP ablation can be either more or less severe. The most severe cases do not develop DCM, but die already between P5 and P10 due to fatigue [80]. Remarkably, MLP-deficient neonatal cardiomyocytes cultured on elastic silicon membranes show no induction of the *in vivo* stress marker brain natriuretic peptide (BNP) and ANF upon pathologic stretch. On the other hand, hormonal stimulation via G-protein coupled receptors still induces these markers [79]. This showed for the first time that chemically and mechanically induced stress trigger different molecular pathways to activate the ANF and BNP mediated stress response. MLP is therefore certainly a key protein in the mechanically induced stress process. In addition, in humans with an α -actinin-2 mutation, the MLP- α -actinin-2 interaction is disturbed, which is also associated with DCM [81]. Conversely, two DCM associated mutations are reported in the MLP gene. These are both predicted to cause significant structural changes in MLP, which may also affect its interaction with α -actinin-2 [79].

Calsarcin-1 seems to modulate mechanical stress, but not isoproterenol induced stress, just like MLP. Unstressed calsarcin-1 deficient hearts are macroscopically indistinguishable from controls, the heart to body weight ratio is unaltered and no fibrosis or myocyte disarray is present. However, the fetal gene program that typically accompanies pathological hypertrophy becomes activated. The established hypertrophy markers ANF and BNP are significantly highly induced, but also MCIP1 (myocyte enriched calcineurin-interacting protein 1), an inhibitor of calcineurin is upregulated. Overexpression of MCIP1 reverses the effects of overexpressed constitutive active calcineurin. Thus, cardiac specific expression of MCIP1 inhibits cardiac hypertrophy, re-induction of fetal gene expression, and progression to dilated

cardiomyopathy that otherwise result from expression of a constitutively active form of calcineurin [82]. When calcineurin activation or pressure overload is induced in calsarcin-1 deficient mice, an exacerbated hypertrophic response is observed [83]. In a wild-type background the overexpression of activated calcineurin leads also to a hypertrophic response, just like the downstream target NFAT [61]. Absence of calcineurin A α does not cause a cardiovascular phenotype [84], but deficiency of calcineurin A β leads to reduced basal heart size and reduced ability to mount a hypertrophic response [85].

The actin-capping protein capZ is also important for sarcomeric integrity. Overexpression of the β_2 -subunit of capZ results in decreased availability of capZ at the Z-disc. Since the overexpressed β_2 -subunit is more easily incorporated in the functional $\alpha\beta$ -heterodimer than the endogenous β_1 -subunit, the balance is shifted towards the $\alpha\beta_2$ -heterodimer. This isoform resides at the intercalated disc and the cell periphery, resulting in decreased availability of the $\alpha\beta_1$ -Z-disc specific dimer [19]. Depending on the expression level of the transgene, a varying decrease of the Z-disc specific isoform is observed. In severe cases this leads to disorganized sarcomeric structure, hypertrophy and juvenile death [19]. Milder reductions of the protein result in increased sensitivity to Ca^{2+} and increased control of maximum isometric tension. PKC β (II) anchorage at the myofilaments is also regulated by capZ [86,87].

Other mouse models of Z-disc components that might give insight in the operation of the stretch sensor at the Z-disc are those for ALP and cypher. In mice, ALP deficiency either yields no phenotype [88] or results in dilated RV cardiomyopathy with onset during embryogenesis (in 15% of the mutant mice, perinatal lethal) or at adulthood (in 85% of the mutant mice) [89]. The latter study reveals a developmental pathway for arrhythmogenic RV dysplasia (ARVD). The differences in defects may be caused by differences in genetic background. In humans, mutations in the ALP gene are possibly associated with ARVD [89].

Cypher deficiency in mice results in neonatal lethality caused by disorganized and fragmented Z-lines [90]. In humans, a spectrum from stereotyped myofibrillar myopathy (MFM) to cardiomyopathy and autosomal dominant muscular dystrophy has been documented [91].

4.2. Mutants of costamere components

Gene ablation of the costameric β_1 integrin gene in mice [92], but also of its partners FAK [92], ILK [93], and Talin-1 [94], respectively, all lead to embryonic lethality between peri-implantation and midgestation due to adhesion defects and cytoskeletal disorganization. As expected, deficiency of these costameric components in cardiomyocyte-specific knockout models result in a less severe phenotype. The mice survive to adulthood but develop heart defects, which suggests that these proteins are implicated also in the development of cardiomyopathies. Ventricular myocyte-

specific ablation of β_1 integrin leads to LV contractility and relaxation problems already at 5 weeks after birth. When pressure-overload is induced by aortic constriction, β_1 integrin mutant mice have a lower survival than control mice. After 6 months, most mutants develop DCM, with fibrosis and disrupted membrane integrity [37]. Cardiac-specific deletion of ILK causes DCM and sudden death at 6 to 12 weeks of age. These mutant mice show disrupted myocyte architecture and extensive fibrosis. ILK ablation in the heart results in a deficient integrin signaling, reduced β_1 integrin protein levels and abrogated FAK activation [48,49]. When FAK itself is inactivated in the heart using a conditional gene targeting approach, the mutant animals develop eccentric hypertrophy after stimulation with AngII or aortic constriction. The heart to body weight ratio is increased and increased LV chamber dimensions are observed. Hypertrophy markers are also upregulated and significant fibrosis is detected in the diseased hearts. In older animals the same phenotype appears spontaneously. Isolated cardiomyocytes of the cardiac-specific FAK mutants are longer, but not wider than control cardiomyocytes. The cells also have disorganized myofibrils [95].

Loss of ILK causes early spontaneous onset of DCM, whereas conditional gene ablation of β_1 integrin or FAK causes DCM in response to an applied cardiac stress (pressure-overload: FAK and β_1 integrin; chemically induced: FAK), suggesting that ILK is an effector of other critical signaling pathways [48]. The costameric partner melusin seems to have a dispensable function in maintenance of basal cardiac homeostasis. The melusin deficient mouse only shows cardiac failure after pressure-overload. Of interest is that, when AngII or adrenaline are used to induce the hypertrophic response in melusin deficient mice, no differences are observed [46]. Hence, the muscle-specific, β_1 integrin-binding protein melusin is, just like MLP, only involved in mechanical stretch sensing.

Germline deficiency of vinculin, another important component of the costamer, results in embryonic lethality with abnormal cardiac development [96]. The heterozygous animals are viable, although they produce decreased amounts of vinculin. In baseline conditions these animals appear fairly normal, only an abnormal ECG and altered intercalated disc morphology are observed. After aortic constriction, vinculin heterozygous mice show an increased mortality rate and the survivors develop cardiac dysfunction 6 weeks post-aortic constriction. The individual cardiomyocytes in the latter mice have misaligned and abnormal myofibrils [97].

γ -filamin deficient mice display neonatal lethality, respiratory failure, reduced skeletal muscle mass, and abnormal skeletal muscle fiber morphology [98]. Clinical mutations in γ -filamin are associated with myofibrillar myopathies [99]. Deficiency in the ubiquitin ligases MuRF3 or MAFbx results in higher probability to cardiac rupture and muscle atrophy, respectively. These conditions are probably the consequence of accumulation of some of their target proteins (γ -filamin, FHL2 and calcineurin) in the cardiomyocytes [100,101].

5. Discussion

The various mutations in Z-disc and costamere components identified through functional studies in animal models or genetic screenings of patients diagnosed with DCM/HCM and heart failure have been particularly instructive in dissecting the cardiac function(s) of these components. They underscore that impairment of these components not only affects the cardiomyocytes structurally but often also interferes with signaling, and that a clear discrimination can be made between chemically and mechanically induced stretch with respect to regulation of the re-induced fetal gene program (ANF, BNP, etc).

However these Z-disc and costameric sensors comprise not the only mechanisms of stretch sensing in cardiomyocytes. So, other mechanisms exist. Besides the mechanical stretch, stretch can also be induced by stretch-activated channels (SACs). SACs, together with volume-activated channels (VACs), belong to the family of mechano-sensitive ion channels. SACs are located in both atrial and ventricular cardiomyocytes and are activated by stretch or strain on sarcolemma. This alters the electrophysiological status of the cardiomyocytes so that the cell can respond appropriately to the mechanical stimulus. Different subgroups of SACs exist that are defined on their ion selectivity. Most of the SACs are cation nonselective, the others are K^+ -selective. A current hypothesis is that the SACs are linked with integrin signaling, which suggests cross-signaling with the costameric stretch-sensor [102,103].

The intermediate filament protein desmin resides at the Z-disc and intercalated discs, the latter can transmit longitudinal stretch. Desmin makes connections between the sarcolemma and the nuclear membrane and it links Z-discs of neighboring myofibrils. Extracellular mechanical stretch alters the intracellular arrangement of the desmin filaments, and this has impacts on cellular behavior. Not surprisingly, mutant desmin mouse models and human mutations in desmin result also in DCM and heart failure, excellently reviewed in [104].

Proteins like PARVB, FHLs and myospryn at the interface between costamere and Z-disc may physically link both stretch-sensors. Hence, both sensors should probably not be considered as isolated entities, but rather as one integrated unit. To further study the relationship between the Z-disc and the sarcomere in stretch sensing, one might consider to make subtle mutations that only affect specific interactions between components while leaving all other functions intact.

Gene ablation of the majority of the stretch-sensor components results in severe disturbed cardiac functions during development or during adulthood (DCM or HCM) but deficiency of several other stretch-sensor components (i.e. zyxin, FLH2, calcineurin, PKC-epsilon, S100A1, etc) is compatible with normal cardiac function. This suggests functional redundancy or compensation by other components or that they are dispensable for cardiac homeostasis under baseline conditions. However, mechanically or chemically induced stress may still cause cardiac defects in these mutant mice.

Very recently, the Z-disc was shown to sequester growth factors of the TGF β /BMP signal transduction pathway like BMP10, and myostatin/GDF8 in skeletal muscle. The interaction of these extracellular ligands with Tcap at the level of the Z-disc, is indicative of a novel action mechanism of these ligands and the Z-disc. The current hypothesis is that they might be released as an adaptive reaction to stretch. In humans a rare mutant of BMP10 that interacts less efficiently with Tcap leads to hypertensive dilated cardiomyopathy due to increased extracellular secretion [17,105].

Although significant progress has been made in deciphering and understanding the stretch-sensors of cardiomyocytes a lot of questions still remain. For example, what is the exact composition of the sensors? When – during development – are the sensors assembled? How does the cross-signaling impact on the different mechanic sensors, and do the latter interrelate with chemical induced stretch processes? How is the contractile state of cardiac myocytes regulated by communication between endothelial cells and their surrounding pericytes? How are stretch sensing, signaling and structural functions of the Z-disc and the costameres interrelated? It will be a challenge in cardiovascular research and pathology to resolve these questions, especially since a better understanding of the stretch-sensor may ultimately lead to better therapy for cardiomyopathy patients.

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