Myofibrillar remodelling in cardiac hypertrophy, heart failure and cardiomyopathies

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BACKGROUND: A wide variety of pathological conditions have been shown to result in cardiac remodelling and myocardial dysfunction. However, the mechanisms of transition from adaptive to maladaptive alterations, as well as those for changes in cardiac performance leading to heart failure, are poorly understood.

OBSERVATIONS: Extensive studies have revealed a broad spectrum of progressive changes in subcellular structures and function, as well as in signal transduction and metabolism in the heart, among different cardiovascular disorders. The present review is focused on identifying the alterations in molecular and biochemical structure of myofibrils (myofibrillar remodelling) in hypertrophied and failing myocardium in different types of heart diseases. Numerous changes at the level of gene expression for both contractile and regulatory proteins have already been reported in failing hearts and heart diseases; these changes are potential precursors for heart failure such as cardiac hypertrophy and cardiomyopathies. Myofibrillar remodelling, as a consequence of proteolysis, oxidation, and phosphorylation of some functional groups in both contractile and regulatory proteins in hearts failing due to different etiologies, has also been described.

CONCLUSIONS: Although myofibrillar remodelling appears to be associated with cardiac dysfunction, alterations in both contractile and regulatory proteins are dependent on the type and stage of heart disease.

Key Words: *Cardiac gene expression; Cardiomyopathies; Congestive heart failure; Myocardial infarction; Myofibrillar alterations*

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries. A sustained increase in hemodynamic function due to physiological activity or pathological stimulus, as well as due to hormonal imbalance or vascular abnormalities, leads to an increase in the size and shape of myocardium (cardiac remodelling). This process involves multiple progressive alterations of subcellular components, including myofibrillar protein assembly, sarcoplasmic reticula, mitochondria, nuclei, cell membranes and the extracellular matrix; these changes contribute to the altered contractile performance of the heart. Subcellular changes associated with cardiac remodelling may be beneficial under certain acute situations, but are often maladaptive and lead to functional decompensation in the long term. However, the key molecular events that determine the transition from

Le remodelage myofibrillaire dans l'hypertrophie cardiaque, l'insuffisance cardiaque et les myocardiopathies

HISTORIQUE : Toute une série de pathologies entraînent un remodelage et un dysfonctionnement cardiaques. Toutefois, les mécanismes de transition entre les modifications adaptées et maladaptées, ainsi ce que les mécanismes de changement de la performance cardiaque entraînant une insuffisance cardiaque, sont mal compris.

OBSERVATIONS : Des études élaborées ont révélé un vaste spectre de changements progressifs dans les structures et la fonction intracellulaires, de même que dans la transduction des signaux et le métabolisme du cœur des divers troubles cardiovasculaires. La présente analyse vise à repérer les modifications de la structure moléculaire et biochimique des myofibrilles (le remodelage myofibrillaire) en cas d'hypertrophie et d'insuffisance du myocarde causées par divers types de maladies cardiaques. De nombreux changements de l'expression des gènes, à la fois dans les protéines contractiles et régulatrices, ont déjà été déclarés en cas d'insuffisance et de maladie cardiaques, et ces changements sont des précurseurs potentiels d'insuffisances cardiaques comme l'hypertrophie cardiaque et les myocardiopathies. Le remodelage myofibrillaire, une conséquence de la protéolyse, de l'oxydation et de la phosphorylation de quelques groupes fonctionnels des protéines contractiles et régulatrices de l'insuffisance cardiaque causée par diverses étiologies, a également été décrit.

CONCLUSIONS : Bien que le remodelage myofibrillaire semble être associé au dysfonctionnement cardiaque, les modifications des protéines contractiles et régulatrices dépendent du type et de la phase de la maladie cardiaque.

compensated heart to cardiac failure are not known. It is believed that many of these changes are reversible, and thus, reverse remodelling may be associated with partial functional recovery. The myofibrillar apparatus is of critical importance in the process of subcellular remodelling (1-3) because contractile proteins are the final effectors of cardiac contraction and are considered to undergo marked endogenous changes themselves during the development of heart failure (HF). The purpose of the present article is to review changes in myofibrillar proteins that may be associated with cardiac dysfunction in different pathophysiological conditions, such as cardiac hypertrophy, cardiac failure and cardiomyopathies. Although changes in myofibrils have also been identified in ischemic heart disease, no effort was made to include discussion on this topic.

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MYOFIBRILLAR ASSEMBLY IN CARDIAC HYPERTROPHY

During a prolonged increase of workload, the heart reacts and increases in size (cardiac hypertrophy), a reaction that represents an adaptive response to normalize wall stress and compensate for the increased hemodynamic load. When the workload is chronically elevated, compensated cardiac hypertrophy progresses to HF, and cardiac dysfunction becomes apparent (4). The importance of molecular changes related to cardiac hypertrophy resides in the fact that key alterations, which determine the transition from compensated hypertrophy to HF, may already be present during the hypertrophic stage. Cardiac hypertrophy results from an imbalance of protein synthesis and protein degradation. According to the current concept, this change in phenotype does not simply mean more of the same or the reactivation of the fetal gene program, but involves complex quantitative and qualitative changes in the contractile machinery, subcellular organelles, myocardial metabolism, cellular signalling and gene expression. However, the characteristic changes in myofibrils in compensated heart dysfunction are not well described, especially in humans, most likely due to the lack of appropriate methods, and for ethical reasons. The available human data are derived from studies of patients with valvular disease and heart malformation; however, most of our knowledge is based on animal models for postmyocardial infarction (post-MI), pressure and volume overload, as well as salt-sensitive spontaneous hypertension rats.

In experimental rat models, hypertrophic stimuli induce an early expression of fetal genes, including those for alpha-skeletal actin (α -SKA) and alpha-smooth muscle actin (α -SMA) (5-7). Increased α -SKA expression was also observed in hearts with compensatory hypertrophy after MI (8) and in cardiac hypertrophy associated with dilated cardiomyopathy (DCM) (9). Increased α -SKA expression in the hypertrophic heart comes hand in hand with an altered protein distribution pattern, which is clearly different from that of normal adult ventricular myocardium (10). The increase in the α -SKA protein level is mirrored at the mRNA level as well, and correlates with the degree of myocyte hypertrophy (9). Suurmeijer et al (10) hypothesized that this fetal actin isoform is required to achieve a high degree of myocardial contractility. This view is consistent with an earlier observation by Hewett et al (11), who found that α -SKA mRNA is associated with increased cardiac contractility. Therefore, an increase in α -SKA is believed to be a programmed response of a diseased heart that is unable to meet the demand for blood supply to different organs in the body (12,13). Because α -SMA is not expressed in the healthy adult myocardium (10,14), the significance of the appearance of this isoform during cardiac hypertrophy in some animals cannot be clearly explained.

Atrial myosin light chain 1 (ALC₁) is normally found in the developing heart and skeletal muscle (15). Therefore, this protein has been designated the embryonic myosin light chain (MLC) (16). The expression of this isoform was detected in the hypertrophied human ventricle (17). The hypertrophied right ventricle of children with tetralogy of Fallot (18) and the hemodynamically overloaded left ventricle of patients with valvular disease (19,20) also express a large amount of ALC₁. It appears that these changes are potentially reversible because surgical intervention with subsequent normalization of the hemodynamic state decreases ALC₁ expression (20). The functional role of ALC₁ appears to lie in increasing the cycling

kinetics of cardiac cross-bridges, thus regulating the contractile state of the heart (21,22). Because ALC₁ is the fast contracting isoform and improves contractility, its re-expression in myocardium is most probably considered to be a part of the adaptive response during cardiac hypertrophy (23).

A significant decrease in myofibrillar ATPase activity is one of the characteristics of HF (24); however, this decrease has been found during compensated hypertrophy. Analysis of myosin heavy chains (MHC), the motor proteins with intrinsic ATPase activity, is one of the most implicit ways to find changes of MHC composition in the hypertrophied heart. In this regard, it should be pointed out that different MHC isoforms, which are expressed in the myocardium, differ in structure and magnitude of ATPase activity in a species-dependent manner. α -MHC promotes faster shortening velocity of cardiac myofibres due to its high ATPase activity (6,25,26), and a heart with a predominant α -MHC content has lower ventricular systolic resistance and higher intrinsic capacity. On the other hand, β -MHC is expressed in the embryonic/fetal ventricles of all mammals, but disappears soon after birth in small mammals and is exclusively replaced (at approximately three weeks of age) by α -MHC. In adulthood, however, β -MHC becomes re-expressed, accounting for 10% to 15% of the total MHC pool. In larger mammals, such as rabbits, dogs, pigs and humans, β -MHC remains the predominant ventricular isoform throughout adulthood (23,27-29). β -MHC has a low ATPase activity and produces cross-bridge force with less energy consumption (30). Therefore, increased expression of genes that encode the slow β -MHC isoform could explain the decreased myofibrillar ATPase activity of hypertrophied myocardium.

Several investigators have reported an increase in β -MHC and a decrease in the α -MHC isoform in the hypertrophied ventricle in rats (31,32) and humans (33), and found that the expression of β -MHC at the mRNA level is closely correlated with the degree of cardiac hypertrophy (31,34). However, during MHC expression, transcriptional activity does not necessarily reflect mRNA or protein levels. Although the α -MHC mRNA level is directly correlated with its nascent pre-mRNA, the β -MHC pre-mRNA and mRNA data are paradoxical (35). This supports the concept that although the regulation of α -MHC takes place mainly at the transcriptional level, the regulation of β -MHC is more complex and post-transcriptional factors seem to come into play. However, the results of Ojamaa et al (31), obtained from cardiac hypertrophy models, are not in agreement with previous findings (35). It was suggested that β -MHC expression is regulated by a transcriptional mechanism, whereas α -MHC expression is under post-transcriptional control. The authors' rigorous analysis of MHC gene expression, when gene transcription (promoter) activity and mRNA level were compared, ascertained that the regulation of the MHC genes largely depends on the model of cardiac hypertrophy. It was found that the expression of α -MHC was decreased and that of β -MHC was increased in both the aortic constriction-induced (pressure loading) and the heterotopic heart transplantation-induced (unloading) cardiac hypertrophy models. While increased β -MHC mRNA resulted from increased transcription in both models, the change in α -MHC mRNA was the consequence of altered transcription activity in the latter case, and post-transcriptional factors, such as altered RNA-processing, mRNA stability and turnover rate, were responsible in the former case. Although the mechanism that leads to changes at the protein level needs further

TABLE 1
Changes in myofibrillar protein composition during development, progressive overload and heart failure as indicated by isoform content

Protein isoform	Fetus, neonate	Adult	Mechanical overload, heart failure
MHC			
Ventricular	$\beta \gg \alpha$	$\alpha > \beta$ (rats) $\beta \gg \alpha$ (humans)	$\alpha \sim \beta$ (rats) $\beta \gg \alpha$ (humans)
Atrial	$\alpha \gg \beta$	$\alpha \gg \beta$ (humans)	$\alpha \sim \beta$ (humans)
Actin	α -SKA $\sim$ α -CA	α -CA $>$ α -SKA	$\uparrow$ α -SKA; $\uparrow$ α -SMA (rodents) $\uparrow$ α -SKA (humans)
MLC			
Ventricular	ALC $\sim$ VLC	VLC $\gg$ ALC	VLC $>$ ALC $\downarrow$ VLC ₂ phosphorylation
Atrial	ALC $\sim$ VLC	ALC $\gg$ VLC	ALC $>$ VLC
cTnT	cTnT ₄ $\gg$ cTnT ₃	cTnT ₃ $>$ cTnT ₄	$\uparrow/\downarrow$ cTnT ₄

$\gg$ Predominance of isoform; $\sim$ Close approximation of isoform's content; $\downarrow$ Decrease of isoform; $\uparrow$ Increase of isoform; ALC Atrial light chain; CA Cardiac actin; cTnT Cardiac troponin T; MHC Myosin heavy chain; MLC Myosin light chain; SKA Skeletal actin; SMA Smooth muscle actin; VLC Ventricular light chain

clarification, upregulation of β -MHC and downregulation of α -MHC in cardiac hypertrophy are noncontroversial in these models. However, rabbits developed no measurable change in the myofibrillar ATPase activity, and only a slight shift in MHC isoform was detected during compensated pressure overload (36). To summarize, a diverse myosin isoform composition may be required to meet the physiological demands for a large number of optimal combinations between the power output and the corresponding shortening velocity to efficiently perform the cardiac contraction-relaxation cycle (23). Furthermore, the increased expression of genes encoding the slow β -MHC isoform subserves as an adaptation during the development of cardiac hypertrophy.

MYOFIBRILLAR ASSEMBLY IN CONGESTIVE HF

There are four basic mechanisms for HF: conditions leading to the development of pressure or volume overload; conditions causing abnormal cardiac muscle contraction and relaxation; conditions limiting ventricular filling; and conditions causing tachycardia or bradycardia (4,6,37,38). In each case, the myocardium compensates for the primary defect through different mechanisms before the stage of overt HF develops. The molecular changes in HF have been studied extensively, and it is presently accepted that a reduction in the relative amount of myofibrils observed in failing hearts is not sufficient to explain the pathophysiology of HF. In this regard, it should be pointed out that changes in the contractile apparatus in cardiac hypertrophy result from qualitative and quantitative alterations in the gene expression program, which is determined by physiological demands (Table 1) (39). These physiological demands are transmitted by a panoply of stimuli, including mechanical loading, catecholamines, hormones, growth factors, cytokines and vasoactive peptides. These result in individual phenotypic responses that are characterized by gene expression pattern, cell morphology and cellular function via different intracellular signalling pathways (23,39-41).

TABLE 2
Alterations of contractile and regulatory proteins in failing human and animal hearts

Protein	Human heart failure	Animal heart failure
MHC	LV, atria: $\uparrow$ β -MHC, $\downarrow$ α -MHC	LV: $\uparrow$ β -MHC, $\downarrow$ α -MHC
MLC	$\uparrow$ ALC ₁ content in left ventricle	$\downarrow$ MLC ₂ phosphorylation
Actin	$\uparrow$ α -SKA	$\uparrow$ α -SKA, α -SMA
cTnI	$\downarrow$ phosphorylation	$\downarrow$ phosphorylation
cTnT	Re-expression of fetal cTnT (cTnT ₄)	Re-expression of cTnT ₄
Titin	Molecule degeneration, isoform switch	No change

$\downarrow$ Decrease; $\uparrow$ Increase; ALC Atrial light chain; cTnI Cardiac troponin I; cTnT Cardiac troponin T; LV Left ventricle; MHC Myosin heavy chain; MLC Myosin light chain; SKA Skeletal actin; SMA Smooth muscle actin

Both α - and β -MHC isoforms are present in the hearts of small mammals, and their expression alters under different pathophysiological conditions in a species-dependent manner (Table 2). However, the expression of proteins is not always regulated in the same way; in fact, it appears to respond individually to different stimuli in different species. The first described biochemical change in failing human hearts was a decrease in ATPase activity (24), which was believed to be associated with decreased contractility. In small animals such as rats and mice, chronic work overload of the ventricles leads to a transition from the dominant α -MHC isoform to the β -MHC isoform; this decreases the cycling rate and shortening velocity of cross-bridges. The abundance of β -MHC in response to work overload may produce the same absolute muscle tension and displacement, but at a slower rate (42,43). Many investigators have shown that MHC isoform shift is accompanied by decreased ATPase activity in different animal models (44-46). It has been suggested that the decrease of enzymatic activity is associated with altered subunits of the myosin molecule, including that subunit responsible for ATPase activity. The formation of such a molecule in the overloaded heart reduces contractility by lowering the rate of interaction between the actin and myosin filaments. However, this concept is not generally accepted, especially due to controversial data obtained from the diseased human heart (19,33,47-51).

In larger mammals, including humans, β -MHC remains the predominant ventricular isoform throughout adulthood, so the heart is already in low gear and there is little potential for a change compared with the smaller mammals (23,30,52). Although normal human myocardium has been reported to contain about 70% β -MHC and about 30% α -MHC at the mRNA level (49,53), protein contents for these molecules do not correlate with the mRNA expression (33,50,53-55) because the corresponding values at the protein level are greater than 95% and 1% to 10%, respectively. The development of HF in humans is linked with a slight increase of β -MHC and a small decrease of α -MHC expression in the left ventricle, so when analysis is performed at the protein level, the failing ventricle contains almost 100% β -MHC and an undetectable amount of α -MHC (28,49,50,53,56). Despite the lack of a remarkable MHC isoform shift, the reported biochemical changes in the myofibrils seem to favour a more economical and efficient myocardial contraction, most likely without affecting Ca^{2+} sensitivity (29,57). It is uncertain whether this

modest change in β -MHC content has any functional significance. Many authors argue against this being a meaningful alteration (26,28,30,33,48,58); however, a recent study on the power output of isolated cardiac myocytes suggested that even a small difference in MHC isoform composition may be significant (59). The study also supported the idea that a critical level of α -MHC content is essential for normal cardiac pump function in humans (59).

The mechanism behind the repression of α -MHC during HF still remains unknown. In several models of cardiac hypertrophy, changes in α -MHC protein content have been shown to be independent of changes at the mRNA level, suggesting the existence of a post-transcriptional mechanism that controls the expression of MHC in response to hemodynamic overload (27,31,50,60). Evidently, data obtained on left ventricles cannot explain the decrease of myofibrillar ATPase activity as a reflection of the slower rate of cross-bridge cycling and myofilament abnormalities, and its function in the failing myocardium. In contrast, α -MHC in the atria normally comprises approximately 90% of the total MHC, whereas the relative amount of α -MHC decreases and is replaced by β -MHC, which increases up to 50% or more during mechanical overloading of the heart (23,52). It has been shown that this isoform shift reduces contractility in the human atrium (55,61) and likely, to a lesser extent, also in the failing human ventricle (49). Regardless of the possible role of a small MHC isoform switch in the failing human myocardium, available evidence indicates that non-MHC isoform-mediated alterations significantly contribute to both the depressed myofibrillar ATPase activity and the mechanical consequences of myofilament remodelling in the failing myocardium (62). Moreover, some investigators have found that the myosin ATPase activity is similar in failing and nonfailing hearts, and thus, this fact casts doubt on the idea of an MHC isoform shift being a meaningful mechanism for explaining cardiac dysfunction in HF (29,33,58).

Because thick filament changes alone seem to be insufficient to explain contractile dysfunction, it was suggested that changes in thin filaments and thin filament-associated proteins may also be involved in the myofibrillar remodelling process (29,63). The relative amount of the actin isoforms and their mRNA levels vary with age, species and pathological situation, including HF (Table 2) (64). As in cardiac hypertrophy, both in vivo and in vitro experimental models of HF showed early expression of fetal genes and their mRNA for α -SKA and α -SMA proteins (65-69). Interestingly, human HF is not associated with cardiomyocyte α -SMA expression: α -SMA appears limited to fibroblasts of postinfarction scars (10). A recent report indicates that, unlike MHC, the α -SKA to α -cardiac actin (α -CA) ratio at the protein level closely follows the changes of their respective mRNA levels (13). The diffuse pattern of the α -SKA positive fibres, which dramatically increase in number, is evident in the failing myocardium and becomes more intensive at the advanced stage (10). α -SKA may be more related to contractile function in the heart compared with α -CA because there is a significant functional correlation at the protein and mRNA levels between the α -actin isoform pattern and cardiac contractile function (10,70,71). However, enhanced expression of α -SKA was associated with increased myocyte stretch, increased wall stress and pressure overload (10). It should be pointed out that data in literature regarding changes in the α -SKA expression are

also controversial. Some investigators have reported a diffuse expression of α -SKA mRNA in the left ventricular endomyocardial biopsies in seven of 14 patients with dilated cardiomyopathy (72), while others have failed to show any quantitative change in the α -SKA mRNA expression in left ventricular samples of the failing heart with various types of cardiomyopathies (73). It is hard to explain this discrepancy; however, it is possible that endocardial biopsy specimens are not representative of the entire ventricular wall. Nonetheless, changes in the distribution of actin isoforms provide new insight into the mechanism by which the heart adapts to increased work overload (10).

Alterations in the expression of MLC have also been considered to play a role in determining the contractile properties of the failing myocardium. Although ALC_1 becomes re-expressed in the overloaded human ventricle (17,19,20,22,74), β -MHC may combine with either ALC_1 and/or ventricular light chain 1 (VLC_1) to form two homodimers and one heterodimer in diseased human ventricles (23). The main consequence of ALC_1 re-expression in the ventricle is the increase of Ca^{2+} sensitivity of the contractile apparatus (74). In this way, ALC_1 modulates ventricular contractility by increasing the cross-bridge kinetics of the β -MHC isoform. β -MHC, in combination with ALC_1 , becomes a faster isoform and at the same time, it improves the efficiency of cardiac contraction (22). The presence of ALC_1 can be seen as an advantage even in HF because it allows for a faster and larger force development at the decreased systolic Ca^{2+} levels under higher heart rate conditions (75). Thus, it appears that a change in the expression of MLC subunit isoforms due to the re-expression of ALC_1 may be responsible for the altered contractility of the failing human ventricle. On the other hand, the expression of ALC_2 was not detected in normal developing (76) or diseased human ventricles (19,20). Nevertheless, it is now well established that in rodent ventricles, there is an MHC isoform shift, rather than a change in MLC expression by hormonal, developmental or hemodynamic alterations, whereas in humans, changes in MLC isoforms (particularly ALC_1), and not those of MHC, seem to regulate ventricular contractility (Table 2) (22,74). It should be noted that the level of ALC_1 is much lower at the end stages of HF (New York Heart Association classes III to IV) than at the earlier stages (New York Heart Association classes I to II) (77). This may represent the loss of compensation provided by ALC_1 and suggests that the loss of ALC_1 re-expression is one of the factors responsible for progression to end-stage failure.

It has been observed that thin filaments reconstituted with troponin from the failing heart have a lower sliding speed of activating Ca^{2+} concentration, but exhibit higher Ca^{2+} sensitivity and show correlation with myofibrillar ATPase activity (78,79). With respect to isoform variation, cardiac troponin T (cTnT) is the only troponin subunit that undergoes modulation of isoform expression in HF (80,81). Although TnT itself does not bind Ca^{2+} , its allosteric effect on the troponin I (TnI)/troponin C (TnC) complex influences Ca^{2+} sensitivity for tension development, depending on the concentration of Ca^{2+} (82). The human heart expresses four TnT isoforms (cTnT₁, cTnT₂, cTnT₃ and cTnT₄) at the mRNA and protein levels; these are generated by an alternative splicing mechanism of the TnT gene (83-85). Isoform appearance is developmentally regulated: cTnT₃ predominates, while cTnT₄ is a minor (fetal) isoform in the adult human heart; the additional two fetal isoforms, cTnT₁ and cTnT₂, are also present, but are

barely detectable (86,87). Different studies have suggested that a significant amount of cTnT₄ is re-expressed in failing myocardium (84,85,87), which exhibits decreased myofibrillar ATPase activity (80,81,83) and a decrease in Ca²⁺ sensitivity (88,89). Changes in the variable region of the cTnT molecule can alter interactions in the troponin complex; this may explain how a shift in cTnT isoforms alters myocardial contractility and Ca²⁺ sensitivity of the cardiac contractile proteins (66,86). All these facts support the concept that a shift in cTnT isoforms toward a more fetal pattern accounts for functional alterations in both human and experimental failing myocardium. However, Solaro et al (90) observed an increase in fetal cTnT in only 10% of the examined samples. Studies undertaken by Knott et al (79), Mesnard et al (86) and van der Velden et al (91) also failed to show any correlation between the pattern of cTnT isoform expression and failing myocardium, suggesting that increased cTnT₄ expression may not be a general characteristic of left ventricular failure. The controversy regarding the role of cTnT isoform changes in HF is further evidenced by results showing both increased and decreased expression of cTnT₁ in the failing heart (85,87).

Different structural proteins have also been shown to undergo changes in their expression in HF. Titin is a huge interface molecule that plays a key role in the assembly of structural and regulatory proteins of myofibrils (92-94), and thus, it determines the active and passive mechanical properties of the sarcomere (95-97). In myocardial tissue, two titin isoforms, a longer but more compliant N2BA isoform and a shorter but stiffer N2B isoform, are coexpressed in a species-dependent manner (98). A difference in the N2BA/N2B expression ratio between the atria and the ventricles, as well as between different layers of the ventricular wall, has also been reported (99,100). The physiological function of titin implies that the loss of titin and/or alterations of its expression may contribute to depressed contractility in the failing heart (101). Some studies have reported degeneration of titin in the failing (102) and the hibernating human myocardium (103). Morano et al (104) observed a decrease in protein content and mRNA level for titin in the failing human hearts; a titin variant with a lower molecular mass ('fast-migrating titin'), probably representing a titin degradation product, was also detected. Others failed to detect a decrease in titin protein content (101), and thus, focused on the connection between long-term alterations in hemodynamic characteristics of advanced HF and titin isoform switch. The relative expression of N2BA titin was found to be increased in the failing heart (N2BA/N2B ratio of 47:53) compared with in the donor heart (N2BA/N2B ratio of 32:68). The increased expression of the longer, more elastic titin isoform represents an adaptation to the increased wall stiffness, but its beneficial role is overridden by an increased expression of desmin and collagen (101,105-107), which are typical characteristics of the failing heart.

Recent results suggest that the difference in Ca²⁺ responsiveness in the failing heart does not reflect intrinsic differences in the protein isoform composition, but rather represents a change in the endogenous phosphorylation status of thin and thick filament proteins (91,108,109). Such an alteration may be determined by the presence of different amounts of kinases, such as the cyclic AMP-dependent protein kinase A (PKA), protein kinase C (PKC) and myosin light chain kinase (MLCK), as well as phosphatases, in the failing myocardium. Fine-tuning of the troponin function in vivo occurs by phosphorylation of some

specific domains of the molecules (110,111), which differ fundamentally from the isoform shifts caused by changes in gene expression. TnI, the inhibitor subunit of the troponin complex, is an important regulatory molecule. It is expressed as a single isoform (cTnI) in the heart, but there is a specific 30-amino acid sequence at the N-terminal of the molecule that contains two PKA phosphorylation sites (at the Ser22/Ser23 or Ser23/Ser24 amino acid residues, depending on species) with functional importance (112). When phosphorylated, cTnI induces conformational changes in the troponin molecules that reduce the Ca²⁺ affinity of TnC and lead to enhanced relaxation (113); this feature is especially beneficial during beta-adrenergic stimulation (2,114,115). The phosphorylation of cTnI by PKA, which is activated via beta-adrenergic receptors, results in a decrease in Ca²⁺ sensitivity of the contractile mechanism (115,116). In HF, the downregulation of beta-receptors and beta-adrenergic signal transduction (117-119) is mirrored at the molecular level as a decrease (56% to 84%) in cTnI phosphorylation (91,111,120,121), which may be responsible for the enhanced myofibrillar Ca²⁺ sensitivity and lower maximal ATPase activity observed in HF (91,110,111,122-124). It was surprising that Knott et al (79) found no change in the cTnI phosphorylation level in failing human hearts. It is possible that the detection method that these investigators applied was not sensitive enough to reveal differences between failing and nonfailing hearts, or that the samples used for analysis may have been from hearts that were not at the advanced stage of failure. The fact that PKA treatment can reverse the aforementioned changes in HF (110,123,124) pinpoints the importance of PKA-mediated cTnI phosphorylation. The upregulation of phosphatases (125,126) in HF, as well as the fact that PKA has a modulatory effect on protein phosphatase-1 activity (127), further emphasizes the crucial role of PKA-mediated regulation in the failing heart.

Complete dephosphorylation of the thin filament was found to abolish most of the differences between failing and nonfailing hearts; this suggests a role for kinases other than PKA (79). An increase in PKC activity was reported in pressure-overloaded (128) and failing hearts (129). These observations are in agreement with findings of beta-adrenergic signalling being upregulated in the failing heart; this system is considered to act via the G-protein-coupled pathway and PKC activation (109). However, the importance of PKC-mediated phosphorylation is obscure because different PKC isozymes are not activated uniformly and may subserve distinct biological functions due to their different substrate specificities. Although cTnI and cTnT are the most probable PKC substrates (130-133), it is not established clearly whether their phosphorylation takes place in vivo conditions. Nonetheless, PKC-mediated phosphorylation of cTnI and cTnT has been reported to have negative effects on myofibrillar function, such as inhibition of maximal MgATPase activity, and to decrease myofilament Ca²⁺ sensitivity, maximal tension development and cross-bridge cycling kinetics, depending on the experimental conditions (131-136). It should also be noted that some PKC isoforms may cross-phosphorylate the PKA phosphorylation sites on cTnI (132,137), which can in turn modify the PKC-specific effects.

The regulatory myosin light chain (MLC₂) is also phosphorylated by PKC, which is stimulatory (138-140), but further studies are needed to confirm this role. Cardiac myosin-binding protein C (cMyBP-C) is a thick filament protein containing

Figure 1) Progression into cardiac hypertrophy and heart failure. It is suggested that relative ischemia and/or hypoxia may be involved in the transition of cardiac hypertrophy to heart failure

accessible phosphorylation sites (141). However, the exact physiological function of cMyBP-C is not fully understood, and it is not clear whether the protein plays a role in the regulation of contraction through phosphorylation (131,142-144). It should be mentioned that Garvey et al (145) have provided evidence against the selective cMyBP-C phosphorylation having any role in modulating contractility. However, recent data suggest that cMyBP-C phosphorylation alters the structure of the thick filament and may modify the kinetics of cross-bridge cycling (146). Furthermore, it may modulate myosin ATPase activity (143,144), but its *in vivo* phosphorylation still needs to be clearly demonstrated.

The net effect of PKC-mediated phosphorylation on myofibrils (TnI/TnT/MLC₂ and MyBP-C) is predominantly an inhibition of the myofibrillar ATPase, because the inhibitory action of TnI/TnT phosphorylation overrides the stimulatory effect of MLC₂ phosphorylation. It should also be pointed out that Suematsu et al (109) have shown that α_1 -receptor- G_q signalling, which is augmented in the failing heart, induces increased myofibrillar Ca^{2+} sensitivity through the Rho-Rho kinase pathway, rather than the PKC pathway. The activation of the Rho-Rho kinase pathway was considered to induce the phosphorylation of MLC₂, which leads to increased Ca^{2+} sensitivity. However, the functional role of this pathway is questionable because MLC₂ phosphorylation was found to be decreased in HF (108,147). Despite conflicting results, the cardioprotective effect of specific Rho-kinase inhibitors on cardiac performance and cardiac remodelling (148) draws attention to a potential regulatory role of Rho kinases in heart function.

Morano et al (149) have reported that phosphorylation of MLC₂ by MLCK increases Ca^{2+} responsiveness of the contractile apparatus. However, the phosphorylation level of MLC₂ was found to be decreased in the left ventricles of failing hearts (108,147), and the contractile response to MLC₂ dephosphorylation was enhanced (108). The latter observation was explained by a difference in the basal phosphorylation of thin and thick filament proteins. Thus, regulation of Ca^{2+} sensitivity via MLC₂ phosphorylation may be a crucial compensatory

mechanism to reverse the detrimental effects of increased Ca^{2+} sensitivity and impaired Ca^{2+} handling on diastolic function in human HF (91,109). A study by Liu et al (150) demonstrated that MLC₂ phosphorylation was markedly decreased in the viable left ventricle, but was increased in the right ventricle and septum of failing hearts in rats after MI. Because the degree of MLC₂ phosphorylation depends on the amount of MLCK present in the tissue, it appears that the observed increase in MLCK content of the failing left ventricle is an adaptive process to compensate for the decreased MLC₂ phosphorylation activity (150).

The increased levels of cTnI and cTnT proteins in the plasma of patients with HF suggest possible proteolytic and/or oxidative damage (151). The degradation of titin (104,106), myosin (50,152) and dystrophin (153), as well as the upregulation of matrix metalloproteinases (154), in HF indicate myofibrillar remodelling. Because apoptotic enzymes are activated during myofibrillar remodelling (155), it was suggested that myocyte apoptosis has a significant contribution to the progressive loss of ventricular function in HF (155,156). In this regard, it should be noted that caspase-3 has been shown to degrade α -actin, α -actinin and cTnT in the troponin complex, resulting in an impaired force/ Ca^{2+} relationship and ATPase activity (157). Although continuously occurring apoptosis of cardiomyocytes has been suggested to be responsible for the transition of compensated hypertrophy to HF (158), the evidence is not exclusive. Furthermore, new findings suggest that apoptosis may offer a window for reversibility of cell damage and cardiac remodelling via the mechanism of 'interrupted apoptosis' (157), because when cardiac cells are dysfunctional due to loss of cytoplasmic proteins, nuclear fragmentation does not develop.

Although it is evident from the foregoing discussion that HF shares some common characteristics with cardiac hypertrophy, the transition from compensated heart dysfunction to HF is still not understood (Figure 1). Exposure to different pathophysiological stimuli induces hemodynamic alterations and increased workload, and thus, the heart is not able to maintain the stroke volume; this leads to an increase in ventricular residual blood volume. The increased ventricular end-diastolic volume and pressure produce myocytic stretch and thereby increase sarcomere length. According to the Frank-Starling mechanism, the increase in sarcomere length improves myocyte contractility, as long as the myocytes are not overstretched. At the same time, adaptation starts at the hormonal level, where catecholamines, renin-angiotensin, endothelin, growth factors and cytokines play a major role (41); these mechanisms enable the heart to maintain cardiac output. On the other hand, the functional changes are accompanied by structural alterations, that is, the development of cardiac hypertrophy in the long run. Initially, cardiac hypertrophy is associated with an improvement in left ventricular function because the myocytes with a qualitatively and quantitatively different contractile apparatus develop a significantly higher contractile force. It is perhaps the relative hypoxia that produces HF in the hypertrophied heart. In this regard, it is pointed out that the hypertrophied myocardium has an increased oxygen demand; however, the adaptation mechanism of the coronary system appears to be unable to keep up with the increased demand. It should also be mentioned that the epicardial coronary arteries are constricted in the hypertrophied muscle fibres (especially during systole), which further aggravates the

already compromised cell metabolism. Eventually, the decreased supply to demand ratio for oxygen may be seen in tissue damage at the structural and functional levels via oxidative stress (159,160), Ca^{2+} overload (159,161) and matrix metalloproteinase activation (162-164). Such changes are considered to explain myofibrillar remodelling and cardiac dysfunction in HF. On the other hand, it may be argued that myofibrillar alterations occur independently of the underlying etiology of HF, and may most likely be a consequence, rather than a primary cause, of the disease (57). Some recovery is achievable, as evidenced by data of patients having undergone left ventricular assist device support (165-169). However, despite impressive hemodynamic recovery, even after the duration of support from a left ventricular assist device, the morphological signs of persistent severe structural damage are evident in patients suffering from HF (170).

MYOFIBRILLAR ASSEMBLY IN CARDIOMYOPATHIES

Cardiomyopathies are defined as diseases of the heart muscle that are characterized by either wall thickening (hypertrophic and restrictive forms) or wall enlargement (dilated form). Cardiomyopathies are associated with cardiac dysfunction, ranging from a symptomless form of HF to progressive HF and sudden cardiac death (171). The etiology of this group of cardiac diseases is very diverse because a wide variety of distinct pathological processes can initiate myocytic changes and myocardial dysfunction (172). Recent studies have shown that in many cases, specific mutations of the sarcomeric proteins associated with myofibrillar remodelling at miscellaneous locations trigger a distinct series of pathological events that remodel the heart; these are known as genetic forms (172). The acquired forms of cardiomyopathies appear as a result of ischemia, hypertension, valvular diseases and chronic viral myocarditis; they may also be induced by toxins, such as alcohol and adriamycin. The remaining group of cardiomyopathies represents an idiopathic form with unknown origin.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is characterized by ventricular hypertrophy, myofibrillar disarray, fibrosis and impaired diastolic function, and is often linked with a high incidence of sudden cardiac death, especially in young adults. Interestingly, most HCM patients have only mild symptoms or no symptoms; however, the remainder suffer from HF, and to a lesser extent, sudden death (173). In 50% of cases, genetic mutation is believed to be responsible for the onset of the disease (171); this group is defined as familial HCM (fHCM), with an autosomal-dominant inheritance and a genetically heterogeneous background. The molecular pathogenesis of fHCM is not completely understood; however, in vitro and in vivo studies have suggested that mutations of sarcomeric proteins are responsible for the hypertrophic phenotype (Table 3). So far, more than 130 mutations in 11 genes encoding for different myofibrillar proteins, including MHC, MLC, tropomyosin (TM), cMyBP-C or troponins, have been identified as being responsible for this disease (39,174-177). Approximately 35% of fHCM cases are attributed to β -MHC, 20% to cMyBP-C, 15% to TnT, less than 5% to α -TM and fewer cases to TnI, VLC₁, VLC₂, alpha-actin, titin and α -MHC gene mutations (12,178,179). The exact mechanism by which the mutations induce hypertrophic transformation is not fully

TABLE 3
Cardiac myofibrillar gene defects associated with hypertrophic and dilated cardiomyopathies

	Number of mutations	Hypertrophic cardiomyopathy		Dilated cardiomyopathy	
		Gene mutation	Risk of sudden death	Gene mutation	Risk of sudden death
Actin	2-12	M	Low	M	Unknown
α -MHC	2	M	Low	—	—
β -MHC	65	M	High	M	High
cTnT	14	M/D	High	D	High
cTnI	8	M	High	M	Unknown
α -TM	4	M	High	M/D	Low
cMyBP-C	30	M/D	Low	—	—
MLC ₁	2	M	Low	—	—
MLC ₂	7-8	M	Low	—	—
Titin	1	M	Unknown	M/D	Unknown
Desmin	1	—	—	M	High
Dystrophin	Unknown	—	—	D	High

cMyBP-C Cardiac myosin-binding protein C; *cTnI* Cardiac troponin I; *cTnT* Cardiac troponin T; *D* Deletion; *M* Missense mutation; *MHC* Myosin heavy chain; *MLC* Myosin light chain; *TM* Tropomyosin

understood, but many mutations appear to impart a diverse array of functional defects (force generation or transmission), including reduced myosin ATPase activity, actin-myosin interaction, cross-bridge kinetics, myocyte contractility and Ca^{2+} sensitivity (23,52,180-182). Therefore, HCM seems to be a reactive adaptation to the underlying disturbance of the contractile apparatus, which is caused mostly by missense mutations or deletions in any of the proteins. According to some investigators, the mutant proteins are polypeptides, which are stable, so they are incorporated in the sarcomere, act in a dominant way and change the function and structure of the sarcomere (183). Because calpains, the Ca^{2+} -activated intracellular proteases, are involved in muscle growth, differentiation and protein turnover (161), it was also speculated that the calpain-mediated degradation of these proteins is decreased due to the mutations, which may lead to an increase in muscle mass and myocardial hypertrophy. However, recent data (184) do not support this hypothesis because protein mutations can lead to truncated proteins that are functionally 'null alleles'; the insufficient production of normal sarcomeric protein would be sufficient to alter the sarcomeric structure and function (185). Some mutations induce functional impairment and support the pathogenesis of a 'hypocontractile' state followed by compensatory hypertrophy, while other mutations induce cardiac hyperfunction with impaired relaxation and determine a 'hypercontractile' state that would directly induce cardiac hypertrophy (185).

Most defects of the β -MHC gene are missense mutations that are associated with extensive cardiac hypertrophy with an early onset and a higher risk of sudden cardiac death compared with other sarcomeric protein mutations (52,186-188). The defective β -MHC is integrated into the thick filament and acts in a dominant negative way that results in a large increase in maximal actin-activated ATPase activity, reduced mechanical efficiency and inefficient ATP use in the myocardial tissue (186,188), but the role of decreased myosin function during fHCM is not explicit. Although β -MHC mutation of some

fibres may be associated with cardiac hypertrophy, it is difficult to indicate how these molecular changes explain the overall hypercontractile state of the heart in this genetic condition (52). A recent study has shown that mutant myosins move actin filaments faster, that is, myosin generates greater velocity with a normal average force (62). Because an individual has both normal and mutant alleles, every muscle cell should have both the normal and faster (mutant) myosin present. Thus, a mixture of two myosin species having substantially different mechanical capacities can lead to internal stress within the muscle cell, which in turn can result in cellular disarray for the occurrence of HF or sudden death. In patients with rare defects of MLC_1 , MLC_2 and α -TM genes, the mutations induce an increase in Ca^{2+} sensitivity and force production (174,189-191), leading to hypercontractile myocardium. Increased expression of ALC_1 was observed in the ventricles of patients suffering from fHCM, partially replacing the ventricular isoform VLC_1 . Recently, an isoform shift from VLC_1 to ALC_1 was found to be correlated with an increase in cross-bridge cycling kinetics (192). Other findings have shown a reduced content of MLC_2 with a concomitant upregulation of MLC_2 mRNA (193,194); this has been described as an inadequate compensatory mechanism.

Transgenic models have shown that altering the TM isoform population or mutating the TM protein in mouse hearts affects cardiac muscle contractility (195,196). A decrease in the α -TM to β -TM ratio (from the normal 49:1 ratio to 1:1), was associated with diastolic dysfunction (197), increased Ca^{2+} sensitivity in skinned fibre preparations (198) and decreased maximal rates of contraction and relaxation in isolated cardiomyocytes (199). A markedly increased expression of β -TM in mouse hearts (α -TM to β -TM ratio of approximately 1:4) leads to the death of animals soon after birth, along with severe cardiac abnormalities in both contraction and relaxation (197). However, the overexpression of the wild type α -TM in the heart does not lead to any morphological or physiological alterations in cardiac performance (200). A potential mechanism for the difference in function between these two isoforms, which are 86% identical, is likely in their interaction with the neighbouring thin filament molecules. Thus, TM isoform shift may account for weaker binding of fast skeletal β -TM to cTnT and may be a major determinant in modulating cardiac muscle performance and regulating Ca^{2+} sensitivity of the myofilaments (196,200). Four mutations of the TM-encoding gene have been described in fHCM so far. The question of why some of these mutations, such as the point mutation (Glu180Val) located near a highly conserved Ca^{2+} -dependent TnT-binding domain, lead to transitions from cardiac hypertrophy to DCM while others do not (201), remains to be answered.

Mutations of the cTnI-encoding gene also evoke fHCM (202) with various phenotypes (ie, asymmetric septal type, ventricular type, apical type). The mutations affect the structurally and functionally distinct regions of cTnI and have a different modulatory effect on contractile force, cardiac troponin C-binding and myosin ATPase activity (203,204). However, most studies have revealed increased Ca^{2+} sensitivity of force production and myosin ATPase activity in certain preparations with fHCM-related cTnI mutants (203-206). It has been shown that both the central inhibitory domain-mutant cTnI (R145G) and the C-terminal region-mutant cTnI (K206Q) induce cardiac hypertrophy. It is surprising that another mutation

in the C-terminal region (G203S) is associated with the 'apical' type of cardiac hypertrophy (202). Therefore, it is hard to explain why mutations with different functional relevance cause myocardial hypertrophy with the same phenotype and vice versa.

The fHCM-causing mutations of the cTnT-encoding gene are associated with a poor prognosis. In experimental studies with transgenic rats (207) and mice (208), the mutation of cTnT has been reported to result in a high incidence of ventricular tachycardia and early sudden death in the absence of overt ventricular hypertrophy. A significant change in cardiac energetics linked to mutation within the TM-binding domain of cTnT was also reported in transgenic mice (208). In this study by Javadpour et al (208), the TM-binding domain of cTnT was shown to be a crucial modulator of contractile function, although it does not directly interact with the ATP hydrolysis site in the myosin head. Takahashi-Yanaga et al (209) found that mutations in cTnT impair multiple processes of Ca^{2+} regulation by troponin, and that there are marked differences in the degree of impairment from mutation to mutation. These investigators also showed that mutations in recombinant cTnT molecules that affect different locations of the molecule (Arg92Gln, Phe110Ile, Glu244Asp, Arg278Cys) can lead to impaired inhibitory function of cTnI, although the change was less pronounced in the presence of the Arg278Cys mutation. Furthermore, Phe110Ile and Glu244Asp mutants potentiated the maximal ATPase activity, which was not observed in the presence of Arg92Gln and Arg278Cys mutants. The high risk of sudden death in patients with cTnT defects was also found to be attributed to altered cardiac energy consumption resulting from an increase in the metabolic cost of force production (210,211). A different type of missense mutation in the cTnT gene, which results in an amino acid exchange of Gln for Arg, was also found to be associated with a clinically severe form of fHCM (208). Several investigators have suggested that variability of the TM-binding domain may explain the differences between skeletal and cardiac thin filament properties (212,213), differences in the properties of cTnT isoforms, and differences in the sarcomere properties of hypertrophic and dilated cardiomyopathic hearts (208).

DCM

DCM represents a heterogeneous group of disorders that are characterized by cardiac dilation and reduced systolic function, which can lead to HF and premature death. Inherited genetic defects are thought to account for 25% to 35% of these cases (171,214). In DCM, 16 chromosomal loci with defects of several proteins involved in the development of the disease were detected. However, the genes responsible for DCM are less well characterized than those for HCM. The mutation or deletion of some myofibrillar and extracellular matrix proteins, such as α -CA, β -MHC, cTnI, cTnT, TM, alpha-actinin, desmin, titin and dystrophin, were observed (Table 3) (215-217). Some studies in knock-out mice and humans have offered insight into the putative role of these proteins in maintaining sarcomere integrity (176,211).

The identification of the mutated actin genes supports the hypothesis that the development of DCM results from impaired anchoring of the sarcomere (12,178). One such defect was located in a shared binding domain for alpha-actinin and dystrophin, which are both associated with Z bands and intercalated discs (218). Therefore, mutations in actin can

lead to DCM if force transmission from the sarcomere to the surrounding syncytium is affected. Moreover, while normal dystrophin stabilizes the sarcomere by attaching the actin cytoskeleton to the extracellular matrix, mutations of dystrophin, which may cause Duchenne and Becker muscular dystrophy in the skeletal muscles (219,220), have also been reported in DCM. The cardiac phenotype of some dystrophin mutations was found to result from an alteration of the promoter region required for specific expression of this protein in cardiomyocytes, or from conformational changes of the dystrophin rod or hinge regions (221). It is worth mentioning that the disruption of the dystrophin complex was also found in viral myocarditis (222,223), which may provide an explanation for the acquired form of DCM.

Additional DCM mutations that cause early-onset ventricular dilation have been identified (28,216,224). A genome-wide linkage analysis on patients suffering from familial DCM by Kamisago et al (216) indicated disease-causing gene mutations in myofibrillar proteins, including β -MHC, cTnT, cTnI and α -TM, which account for approximately 10% of the cases of familial DCM. The investigators found that sarcomere mutations appeared in regions that could be expected to diminish the mechanical function of cardiac myocytes. The myosin mutation site was in the alpha-helix region, which contributes to the tight binding of actin and disrupts stereospecific interactions between myosin and actin. The cTnT deletion involved the domain that is responsible for TnC binding and may also impair contractile function (214,216). Moreover, incorporation of abnormal protein such as cTnT was proposed as the mechanism leading to structural breakdown of sarcomeres and possibly to increased apoptosis (225). The truncated cTnT showed altered stability within the sarcomere and caused misregistration of the Z bands, myofibrillar disarray and degeneration (226). Other studies have revealed that the functional consequence of cTnT mutations is a decrease in Ca^{2+} sensitivity of cardiac muscle contraction and a reduction in the rate of Ca^{2+} -activated actomyosin ATPase hydrolysis and thin filament sliding speed (213,227). Morimoto et al (228) have also recently reported a similar decrease in Ca^{2+} sensitivity in rabbit heart trabeculae when endogenous troponin was replaced with human cTnT (either wild type or mutant). The decreased Ca^{2+} sensitivity, together with the decreased cross-bridge turnover rate and reduced cooperativity, suggests that the expression and incorporation of this DCM-related cTnT mutant *in vivo* may result in myofilaments that are markedly less responsive to Ca^{2+} , contract more slowly and thus are unable to produce sufficient force during activation (213,228).

The molecular mechanism of DCM that is related to a missense mutation in the strong TM-binding region of cTnT (R141W) was studied using a sensitive mass-measuring device (229); this mutation markedly increased the affinity of cTnT for α -TM. Because cTnT structurally integrates cTnI into the thin filaments, cTnT mutation in the TM-binding region strengthens the integrity of cTnI in the thin filament by stabilizing the interaction between cTnT and TM; this may allow cTnI to inhibit the thin filament more effectively, leading to Ca^{2+} desensitization. Such an interdependence of thin filament protein expression suggests that mutations in one of the contractile or regulatory proteins may trigger the misregulation of thin-filament protein expression, resulting in sarcomere loss and myocyte disarray (226,230). Ca^{2+} desensitization may therefore directly lead to reduced systolic function in DCM

and increased systolic function with diastolic dysfunction in fHCM (229). The functional changes caused by cTnT mutation in DCM appear to be opposite from those induced by fHCM-related cTnT mutations. This suggests that the primary mechanism for the pathogenesis of cTnT mutation-associated DCM is a deficiency of force generation, which is in contrast to the enhancement of force generation in fHCM associated with other mutations in cTnT (228). However, the role of Ca^{2+} sensitization/desensitization in myopathies is still not completely understood. Recently, Venkatraman et al (231) reported the Ca^{2+} sensitivity of force development in porcine cardiac fibres using R141W and K210 cTnT mutants known to cause DCM independent of fHCM. These authors concluded that a decrease in Ca^{2+} sensitivity is unlikely to be a common denominator in the pathogenesis of DCM caused by cTnT mutations.

The two major titin splice forms, N2B and N2BA, vary in their expression in pathological states, including cardiomyopathies (187,232). In addition, titin mutations may cause DCM in a subset of patients (232). Two mutations located in the Z line region of titin, Val54Met and Ala743Val, decrease binding affinities of titin to Z line proteins. Two additional mutations were found in the N2B region, one of them presumably encoding for a truncated nonfunctional molecule. The missense and frameshift mutations in the titin-encoding gene were studied by Gerull et al (233). The missense mutation possibly induces the disruption of the highly conserved hydrophobic core sequence of an immunoglobulin fold located in the Z disc-I band transition zone while the frameshift mutation leads to truncated A band titin, inducing DCM. Because titin is the main intracellular determinant of passive tension in myocytes, it was suggested that it may be involved in altered ventricular compliance of the DCM heart, where its amount was markedly reduced (232). However, reduced titin levels in DCM are expected to result in a reduction of diastolic stress, whereas passive tension is typically increased. Based on recent results by Wu et al (100), N2B titin is upregulated in DCM, that is, the N2B/N2BA titin isoform ratio is increased, and it is expected to result in elevated passive stiffness that is consistent with DCM. In an experimental model of pacing tachycardia HF in dogs, it was found that titin-based stiffness was increased; paced animals revealed markedly increased N2B titin isoform expression.

Transgenic mouse models showed that while moderate overexpression of the wild type desmin is not detrimental to the heart, significant changes in phenotype and function are present due to a seven-amino acid deletion (R173 through E179) for the mutation of desmin or R120G point mutation of alpha/beta-crystallin, a small chaperone interacting with desmin (234,235). These mutations induce an overlap of the phenotypes of DCM and HCM, which progress into HF rapidly when accompanied with increased expression of the mutant protein. The mutant proteins most likely act in a dominant negative way and lead to the appearance of desmin-positive aberrant intrasarcoplasmic and electron-dense granular filamentous aggregates, and these aggregates are characteristic of human desmin-related myopathy. The pathomechanism for this entity is not yet fully understood, but the defective desmin protein was seen to result in an impaired force transmission or in contraction-induced cellular damage (215). A genetic analysis of patients suffering from dominantly inherited or desmin-related myopathy revealed that the

mutant desmin interferes with the normal assembly of intermediate filaments, resulting in fragility of the myofibrils and severe cardiac dysfunction (236). The mutations were clustered in the C-terminal part of the rod domain critical for filament assembly.

Based on the mutant protein, it is hard to predict which type of cardiomyopathy will develop. It is believed that protein mutations leading to defective force production (mainly the sarcomeric proteins) typically produce fHCM, while mutations leading to defects of force transmission (mainly the cytoskeletal proteins) typically produce DCM (237). Existing data show that the mutation of a certain protein can induce both fHCM and DCM, and even an overlap in phenotype, depending on the mutation site and environmental modifiers (171,213,216,224,228,234,235,238). Identifying changes that are specifically associated with cardiomyopathies of nongenetic background, such as those due to ischemia or cardiac overload, or those associated with unknown origin (idiopathic) is a challenging task. Many studies (228,234,235,238) have been carried out to this end; however, most hearts were already in the failing stage at the time of examination. Furthermore, most of the described abnormalities in these models of cardiomyopathy are similar to those of HF, and it is difficult to segregate cardiomyopathy-specific changes from the HF-specific ones. Nonetheless, some attempts have been made to clarify this point.

It was observed in patients with DCM with different etiologies that an increased expression of α -SKA mRNA is generally present (9), but this correlates with the degree of myocyte hypertrophy rather than with DCM. For instance, Suurmeijer et al (10) found that idiopathic DCM does not generally exhibit this α -SKA accumulation. However, one can speculate that the absence of α -SKA accumulation in idiopathic DCM means the loss of one of the potential adaptive mechanisms that could help the diseased heart to cope with demand. It is generally believed that changes in MHC isoforms have no functional significance in humans. However, it was found that the improvement of left ventricular function in patients with DCM was directly related to an increase in α -MHC expression (239). These MHC isoform changes, however, are not DCM-specific. The embryonic MLC isoform, ALC₁, was re-expressed in the ventricle of patients with cardiomyopathy, as seen in patients with HF. ALC₁ expression change patterns are thus similar to those of MHC, which are correlated with an improved contractile state and increased Ca²⁺ sensitivity of the contractile apparatus (74).

In addition to the similarities, some differences have been described between DCM and HF. Changes in MLC₂ expression and phosphorylation level are often reported in HF (108,147,150), but these levels are unchanged in DCM (74). Furthermore, TM expression and the cTnI phosphorylation level were also unchanged. In a recent study, Haase et al (194) reported a dramatic upregulation of MLC₂ mRNA, but a decrease at the protein level in idiopathic DCM compared with control and ischemic cardiomyopathy. These investigators suggested that this discordant change can be seen as an inadequate compensatory mechanism and that it is a specific feature of idiopathic DCM. Increased N2B titin expression, which was reported in DCM (100), does not seem to be DCM-specific either. It occurs mostly in the endocardial myocardium and is most likely associated with increased myocardial stiffness. cDNA microarray studies have revealed changes in the

expression of numerous proteins in DCM (217). A set of genes that includes myofibrillar proteins, such as cTnI, TM, β -MHC, α -actinin and α -CA, as well as collagen, atrial natriuretic peptide and heat-shock proteins, became upregulated. However, other genes, such as Ca²⁺/calmodulin-dependent kinase, SERCA2a and others involved in Ca²⁺ signalling, showed downregulation. Their contribution to DCM, however, has not been identified.

CONCLUSIONS

From the foregoing discussion, it is evident that a number of molecular and cellular alterations in myofibrils have been identified in the failing heart and in diseases that are potential precursors to HF. In the present article, we reviewed how changes in gene expression and post-translational modifications of both contractile and regulatory proteins may explain altered myocardial contractility in HF and cardiomyopathy. Intrinsic properties, as characterized by changed levels of expression of particular contractile or regulatory protein genes, are considered to result in the alteration of the molecular and biochemical composition of myofibrils, and this remodelling appears to be associated with cardiac dysfunction. Because ventricular remodelling after MI is considered to play a major role in the progression of HF (240), we concluded that alterations in myofibrillar ATPase activity, myosin isozyme shift and myosin gene expression were associated with cardiac dysfunction due to MI in rats (241,242). All these changes that reflect myofibrillar remodelling were partially prevented by the blockade of the renin-angiotensin system, indicating that the activation of the renin-angiotensin system may be involved in both myofibrillar and cardiac remodelling in this experimental model. This view is consistent with our earlier concept that subcellular remodelling may be associated with cardiac remodelling during the development of HF (243) due to MI. It should be pointed out that myofibrillar remodelling and cardiac dysfunction have also been reported to occur in insulin deficiency and thyroid hormone imbalance (244), and are thus not limited to HF due to MI.

In the past two decades, it has become increasingly clear that genetic factors play a significant role in the pathogenesis of many cardiovascular disorders, and thus, the field of molecular genetics is considered to provide an opportunity to decipher the genetic basis of myofibrillar remodelling in cardiovascular disease. Isolation of some disease-causing genes has already led to the identification of specific protein abnormalities; this identification is essential to elucidate the pathogenesis of, and design specific therapy for, HF. New sensitive and specific methods allow us to determine very slight molecular changes, but the detection of a low amount of protein and the analysis of the genetic database are still a challenge. Although various animal models have contributed to a better understanding in contractile dysfunction, the available human data lag behind the knowledge of myofibrillar remodelling that we gained from those models. Unfortunately, no ideal animal model exists for studying cardiac remodelling in humans due to differences in coronary circulation, infarct location, healing process, neurohumoral pathways and receptor population. Furthermore, genetic manipulation of single genes appears to be incapable of mimicking the human phenotype of the cardiovascular disease. Therefore, extrapolation from animal studies to the human heart is problematic,

and it appears that the cause and effect of myofibrillar remodelling in the progression of HF still need to be elucidated. Likewise, the mechanisms of abnormalities in myofibrillar proteins in HF due to different etiologies remain to be examined. In particular, the signal transduction events leading to myofibrillar remodelling in all types of HF should be established.

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