Proteins of the Contractile and Relaxing Systems in the Nonfailing and Failing Heart

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Abstract

Cardiac hypertrophy is a common result of hemodynamic overload and often precedes development of heart failure in humans independent of the underlying cardiac disease. It is frequently accompanied by changes in the expression of genes encoding for proteins involved in cardiac contraction and relaxation. In small mammals like rodents, qualitative changes in protein isoform expression of myosin heavy chains (MHC), actin and tropomyosin (TM), and quantitative changes such as a decreased expression of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLN) have been described. The adaptive transition from α-MHC to β-MHC, resulting in lowered myosin ATPase activity and an economized contraction, can account for most of the functional alterations observed in hypertrophied rat myocardium. In failing human ventricular myocardium this can not be the case for there is almost no switch in MHC-expression: the β-MHC isoform already predominates in the normal human ventricle. Quantitative changes in the calcium handling proteins (SERCA, PLN, Na^+/Ca^{2+}-exchanger) do, however, appear to be a common feature of the failing human heart. This review will focus on changes in protein expression of the major proteins of contraction and relaxation with a comparison between mammalian models of cardiac hypertrophy and the situation in human failing myocardium.

Key words: heart failure, cardiac gene expression, myosin, contractile proteins, sarcoplasmic reticulum, SR-Ca^{2+}-ATPase, Na^+/Ca^{2+}-exchanger.

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Generally speaking, the two main characteristics of the failing heart are reduced contractility during systole and impaired relaxation during diastole [90]. The failing heart shows an inadequate cardiac output during exercise or rest and elevated intracardiac filling pressures. In most cases, heart failure is preceded and accompanied by cardiac hypertrophy which is understood as a non-specific process of adaptation to increased cardiac pressure or volume load caused by various underlying cardiac diseases [30, 41]. However, the possibilities of this adaptive mechanism are limited by anatomical and biological factors and its consequences are not entirely beneficial. An example for the latter is the reduced ventricular compliance secondary to hypertrophy-associated fibrosis. The symptoms of heart failure occur when the ventricular performance starts failing to meet the hemodynamic requirements despite a compensatory hypertrophy. Thus, cardiac hypertrophy and later heart failure are the common pathway of many different cardiac diseases such as valvular, ischemic, and hypertensive heart disease or cardiomyopathies [57].

Although the clinical signs and symptoms of heart failure have been well known for over a century, investigations of the underlying physiological and biochemical processes in the failing myocardium were begun only in the 1960s: Alpert and Gordon [1] demonstrated a depressed myosin ATPase activity in failing hearts and Spann and coworkers [101] were among the first to precisely describe a decreased contractility in isolated papillary muscle strips from hypertrophied and failing cat hearts showing decreased shortening velocity, length-tension curves and maximum tension development. They concluded "extreme quantitative abnormalities of the intrinsic contractile state of each unit of heart muscle". Since then, the identification of important cellular and molecular alterations in the hypertrophied and failing myocardium has led to a new pathophysiological understanding of heart failure. The rapid improvement of molecular biology techniques over

Actin

The thin filament actin molecule is a 42 kDa globular protein with a helical structure. In mammals and man, the different actin forms are encoded by a multigene family, but a strong overall amino acid sequence homology of more than 90% between the different mammalian actin forms has been identified. There exist two sarcomeric actin forms in the heart, α-skeletal and α-cardiac, and a cytoskeletal form of β-actin is also expressed in the cardiac myocyte (reviewed in [68]). In the rat ventricle, the α-skeletal form is predominant at the fetal and neonatal stage, whereas the α-cardiac actin is expressed in the adult and senescent heart [25, 71]. Pressure overload leads to a reinduction of the fetal α-skeletal actin, but other than the β-MHC, it returns to normal low levels within a few days even with persisting pressure load [55, 56, 98]. Because of the great homology between the two forms of sarcomeric actin and the transitory character of the isoform switch, its functional consequence is still unclear. Two slight differences between the isoproteins in the NH_2-terminus that interacts with the MHC-head might suggest a significance in actin-myosin binding. On the other hand, it is possible that the reexpression of α-skeletal actin only reflects an overall reinduction of a fetal gene program [56]. As for the MHC, it was directly demonstrated by nuclear run-on assays that developmental α-actin expression is transcriptionally regulated [19]. Moreover, after pressure overload-induced hypertrophy, the regulatory pathways for α-actin expression are different and independent from those for MHC: striking experimental evidence for that hypothesis is given by the different time courses of the switches in the encoding mRNA after induction of pressure overload [26, 55, 98] - the α-skeletal actin gene activation occurs earlier than that of the β-MHC gene and is only transitory in nature - and by a difference in the distribution within the heart as shown by in situ hybridization [96].

In the human ventricular myocardium, α-actin expression differs from that in rodent heart. The low fetal expression of α-skeletal actin (about 20%) increases steadily during growth and reaches a steady state of about 60% of total actin in adults [18]. Thus, in human heart, α-skeletal actin is the major form in contrast to the predominance of α-cardiac actin in the myocardium of adult rats. Skeletal and cardiac sarcomeric actin are coexpressed in the human ventricle at a ratio of about 6 to 4 [18, 42, 108] which remains unchanged in human hypertrophied or failing myocardium [18]. However, this does not rule out a transitory switch in human sarcomeric actin expression similar to that observed in rat heart, since usually the cardiac specimens for studies in humans are obtained from patients with chronic cardiac disease many years after the onset of the disease.

Tropomyosin and troponin

Tropomyosin (TM) is a 70 kDa rodlike dimeric protein consisting of two subunits and lies in the groove of the thin filament spanning seven actin molecules. It is associated with the troponin T of the troponin complex (see below). Tropomyosin and the troponin complex form a functional unit regulating Ca^{2+}-activation of the actin-myosin interaction. The troponin complex is composed of its three subunits, troponin C (TnC), troponin I (TnI) and troponin T (TnT). TnC contains the Ca^{2+} binding site. In contrast to fast skeletal muscle with two sites on TnC, cardiac TnC has only one active Ca^{2+} binding site [52, 94]. Cardiac TnI has a direct regulatory function on actin-myosininteraction: first, together with tropomyosin it is an inhibitor of actomyosin ATPase at low calcium levels as during cardiac diastole [113], and second, its phosphorylation by the cAMP-dependent protein kinase A decreases the Ca^{2+}affinity of TnC [52]. TnT is the TM binding subunit.

Two different subunits exist for the dimeric tropomyosin, α-TM and β-TM. They are both composed of 284 amino acids, but are encoded by different genes. Additionally, several isoforms expressed in a tissue specific manner are generated from each gene by alternative splicing and mRNA processing. In the fetal rat ventricle, as in skeletal muscle, both subunits, the α-TM and β-TM, are present whereas in the adult myocardium of the rat only the αα-TM-homodimer is detected (reviewed in [68]). As observed for the fetal α-skeletal actin, pressure overload by aortic constriction induces in the rat heart a reexpression of the fetal β-TM subunit of striated muscle, smooth muscle and nonmuscle tissue, although the functional meaning of that switch is unknown [56]. In larger mammals like cow, as well as in the human myocardium, a small amount of β-TM is present in the fetal as well as in the adult heart without a difference between atrial and ventricular myocardium. Moreover, the relative amount of β-TM increases during development and reaches about 10% in adult human heart [53].

Two different troponin C forms, the fast skeletal and the slow skeletal/cardiac form have been identified which differ in amino acid sequence and the number of Ca^{2+} binding sites (see above), thus contributing to the difference in myofibrillar Ca^{2+} sensitivity between skeletal and cardiac muscle. They are encoded by different genes (reviewed in [68]). Although very little is known about TnC expression during development, hypertrophy or heart failure, it can be assumed that at least no qualitative changes occur, since the cardiac TnC seems to be essential to obtain normal myocardial performance such as stretch-induced enhancement of contractile force [10].

For troponin I, three different tissue specific isoforms for fast skeletal, slow skeletal and cardiac muscle encoded by separate genes have been described in different animal species and man (reviewed in [54]). Only the cardiac form contains a phosphorylation site and has, therefore, the capacity for regulatory fine tuning of actomyosin activation by decreasing TnC Ca^{2+} affinity. Since this phosphorylation is cAMP-dependent, this mechanism was suggested to protect the cardiac myocyte against overstimulation by catecholamines [113]. In fetal rat heart, cardiac and slow skeletal TnI are both detectable at the

mRNA and protein levels with the slow skeletal form completely disappearing around the third post-natal week [84]. Cardiac TnI is the only form expressed in adult rat heart, and pressure overload by aortic banding does not alter TnI expression [54]. A similar developmental regulation of TnI as in rat heart is observed in human hearts. During the fetal period, slow skeletal TnI is present in the heart, but in adult heart, only the cardiac form is detectable [54] with no marked difference between atrial and ventricular myocardium [53].

Troponin T is encoded by a multigene family with different genes for fast skeletal TnT, slow skeletal TnT and cardiac TnT. Each gene allows the expression of several alternatively spliced mRNAs which are translated into up to 5 different isoforms for each, skeletal and cardiac TnT respectively (5, reviewed in 68, 75). Although the number of cardiac TnT isoforms expressed in myocardium may vary with different species, it is discussed controversially in the literature: in adult rat heart, only one cardiac TnT form exists whereas in fetal heart a second, embryonic form is expressed, its expression decreasing upon cardiac maturation. In the rabbit myocardium, however, five cardiac TnT isoforms have been detected by immunological techniques with one form predominant in embryonic and two predominant in adult heart [5]. In contrast, in bovine heart, only two cardiac TnT isoforms have been isolated [106, 111]. Independent of species-differences, the regulation of cardiac TnT expression varies with the developmental stage. In contrast to β-TM, β-MHC or α-skeletal actin, no reexpression of the fetal cardiac TnT isoform was observed in pressure overloaded rat heart [68]. In human fetal heart, four TnT isoforms have been characterized by Western blotting. According to their mobility in gel electrophoresis, they are referred to as TnT1 through TnT4, numbered in order of decreasing molecular size [3, 4]. In adult human myocardium, two cardiac TnT isoforms with different sizes, which correspond to the fetal forms named TnT3 and TnT4, are present. Unfortunately, the nomenclature in the literature is somewhat confusing since the two adult human cardiac TnT forms, today called TnT3 and TnT4, were originally termed TnT1 and TnT2, respectively. TnT3 is the predominating form in adult human myocardium whereas TnT4 is expressed at a lower level than in the fetal heart [3, 4].

In the human failing heart, almost nothing is known about alterations in the expression of tropomyosin, troponin C or troponin I. Concerning troponin T, there is evidence for a slight upregulation of one of the two cardiac TnT isoforms present in adult human myocardium, TnT4, as shown by Western blotting: while its relative amount of total cardiac TnT is only 4% in nonfailing left ventricular myocardium, it increases significantly up to 12% in terminally failing hearts [4]. As Tobacman and Lee [106] demonstrated using isoform reconstitution assays, the two adult bovine TnT isoforms affect the myofibrillar ATPase activity *in vitro* differently, thus suggesting a functional consequence of a altered TnT isoform pattern as observed in failing human heart. Indeed, in a study by Anderson and coworkers [4], a negative correlation between myofibrillar ATPase activity and TnT4 content was reported. The authors concluded, that TnT alterations might in part be responsible for the reduced myofibrillar ATPase activity detected in terminally failing hearts [91]. However, in a study recently published by Mesnard and colleagues [75], no significant differences between failing human myocardium and control hearts in the amount of mRNA corresponding to the two TnT isoforms present in adult human myocardium could be detected by RNAse protection assays.

Sarcomeric disorganization in failing human heart

In addition to the alterations of individual contractile proteins described above, several morphologic changes concerning the quantity and organization of the sarcomeric structures in the terminally failing human heart have been observed. After the initially beneficial hypertrophy response of the myocardium, chronic overload causes deterioration of myocardial cells probably due to a chronic energy deficit in the hypertrophied cell. Decreased capillary density in the hypertrophied heart, greater diffusion distances and a relatively lower number of mitochondria per myofibril in the hypertrophied cardiomyocyte contribute to that deficit (reviewed in [57]). Using monoclonal antibodies, a significant reduction in the amount in myosin, actin, tropomyosin troponin T, and the structural protein titin, together with severe disarrangement was detected in terminally failing myocardium [48]. Taken together with the abnormalities in calcium handling proteins described below, this may in part contribute to the reduced contractility of failing human myocardium [46, 100].

Proteins of calcium transport: the major regulatory tools of contraction and relaxation

The central role of Ca^{2+} in regulating muscle contraction is well established. Upon depolarization of the sarcolemma, extracellular calcium enters the cells through voltage gated Ca^{2+}-channels (dihydropyridine receptor), located on the transverse tubules (T tubules) formed by the sarcolemmal membrane. This triggers the release of Ca^{2+} stored in the sarcoplasmic reticulum through the ryanodine receptor (RyR). The sarcoplasmic reticulum (SR) is an intracellular membrane network constituted of two main components: the junctional region with the terminal cisternae containing the RyR in close contact with the sarcolemmal T tubules, and the longitudinal region surrounded by the myofibrils. This longitudinal region contains the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SR Ca^{2+}-ATPase, SERCA) and its regulatory protein phospholamban (PLN) (see figure 1). When the cytosolic calcium levels increase, calcium binds to troponin C which initiates the contraction (see above). Myocardial relaxation is mainly triggered by calcium reuptake by the SR: the SR Ca^{2+}-ATPase pumps cytosolic calcium into the lumen of the SR where it is bound by the storage protein calseques-

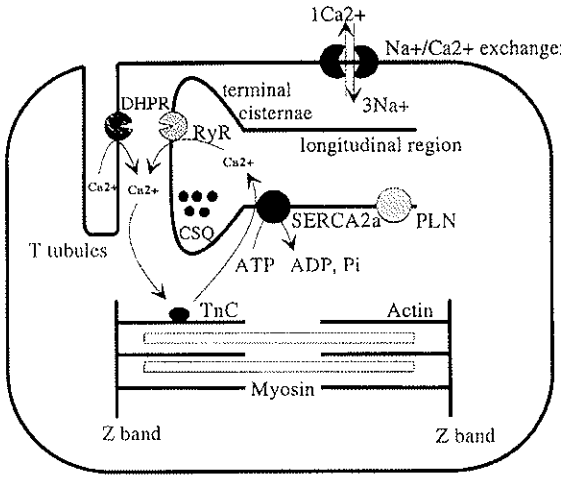

Figure 1. Schematic representation of a cardiomyocyte with the contractile apparatus and the Ca^{2+} handling proteins. DHPR: dihydropyridine receptor; RyR: ryanodine receptor; CSQ: calsequestrin; SERCA2a: cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase; PLN: phospholamban; TnC: troponin C; T tubules: transverse tubules.

trin. This rapid decrease in cytosolic free calcium concentration leads to a dissociation of calcium from troponin C, causing a conformational change in the troponin-tropomyosin complex that inhibits actin-myosin binding (reviewed in [8, 38]). The reuptake by the SR Ca^{2+}-ATPase contributes to about 70% of the calcium flux during relaxation, this protein is therefore regarded as a key player in cardiac relaxation. Moreover, since cardiac contraction is triggered mainly by calcium release from the sarcoplasmic reticulum, its filling state also determines the force of contraction generated during systole. In the heart, the remainder of the calcium transported during diastole is moved outside the cell across the sarcolemmal membrane, mainly by the Na^{+}-Ca^{2+}-exchanger [14, 15, 102].

Sarco(endo)plasmic reticulum Ca^{2+}-ATPase and phospholamban

The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) represents up to 40% of the total protein in cardiac SR [104]. Five distinct protein isoforms have been identified encoded by three independent genes (reviewed in [8]). The SERCA1 gene encodes two alternatively spliced and developmentally regulated transcripts : the SERCA1a isoform is expressed in adult fast-twitch skeletal muscle, the SERCA1b isoform is transiently expressed in neonatal fast skeletal muscle. The SERCA2 gene also encodes two alternatively spliced isoforms of the Ca^{2+}-ATPase mRNA expressed in a tissue specific manner in rats, rabbits, pig and human: the cardiac slow-twitch skeletal isoform SERCA2a is present in heart at very early stages of development, its expression is steadily increasing throughout development, and SERCA2a is the predominant isoform in adult myocardium (reviewed in [8, 67, 76]). SERCA2a is also present at the onset of differentiation of skeletal muscle [6]. SERCA2b is a housekeeping isoform present in all cellular types at low levels but mainly in smooth muscles and in non-muscle tissues. The SERCA3 gene encodes an isoform that is expressed in a wide variety of tissues. The activity of cardiac SERCA can be modulated *in vivo* by the phosphoprotein PLN [103] also located on the SR longitudinal region: unphosphorylated PLN inhibits SERCA activity by interacting with the enzyme and decreasing its affinity for calcium. PLN can be phosphorylated by a panel of cAMP-dependent and independent protein kinases (reviewed in [67]). In its phosphorylated form, PLN is unable to bind to SERCA which results in an increase in the ATPase activity and calcium uptake by SERCA. PLN is encoded by a single gene that generates multiple transcripts of different sizes: this has been shown in dog heart [36], rabbit heart [35] and in the mouse heart [37].

Alterations in SERCA expression and calcium reuptake by the SR have been extensively studied in hemodynamically overloaded hearts of rat, guinea-pig and rabbit: left ventricular hypertrophy was induced by aortic banding [13, 34, 63], or right ventricular pressure overload by placing a constrictive clip around the pulmonary artery [86]. Depending on the degree or duration of overload and on the type of surgical intervention, hemodynamic loads produce a wide range of hypertrophic responses from mild compensated cardiac hypertrophy to decompensated end-stage heart failure. In small mammals, it could generally be demonstrated, that the expression of cardiac SERCA2 is decreased at the mRNA level as well as at the protein level, these levels correlating inversely with the degree of hypertrophy and heart failure. It is important to note that in contrast to the qualitative changes observed for MHC-expression, only quantitative changes of SERCA expression occur and no other SERCA isoforms were detected. At the functional level, the decrease in SERCA expression seems to be well correlated with a diminished SR calcium uptake rate (reviewed in [8, 64, 67]). In mild cardiac hypertrophy, no alterations were observed either at the protein level or at the mRNA level in banded animals related to sham-operated animals [13]. In more severely overloaded and hypertrophied rat hearts, a constant quantitative decrease of SERCA2a mRNA-levels with time course, ranging from -18% 4 h after aortic banding up to -68% after 1 month, has been detected. This decrease correlated with an increased degree of hypertrophy, a decreased protein level and a decreased SR Ca^{2+} uptake with time after operation [59]. Feldman and coworkers [34], using a subacute aortic banding hypertrophy model, observed that the SERCA2a mRNA was decreased by 50% in animals demonstrating obvious clinical signs of heart failure (20-week failing left ventricular hypertrophy) related to age-matched controls. In 20-week nonfailing left ventricular hypertrophy, there was a notable absence of a change of the SERCA2a mRNA level in the left ventricle. These authors have then proposed that the decrease in SR

Ca^{2+}-ATPase mRNA could be a marker for the transition from compensated hypertrophy to heart failure in these animals. One study using a rabbit pulmonary artery banding model reported an additional decrease in PLN mRNA corresponding well to the simultaneously diminished SERCA2a mRNA [86].

Another model of interest to analyse abnormal Ca^{2+} handling in failing hearts is the hereditary cardiomyopathic syrian hamster. The BIO 14.6 strain displays a thickened ventricular wall resembling hypertrophic cardiomyopathy (HCM). A recent study demonstrated that in this model there is also a decrease of sarcolemmal and sarcoplasmic Ca^{2+}-ATPase activities compared with age matched controls accompanied by a decreased level in their encoding mRNA levels [62]. Heart failure induced by rapid right ventricular pacing of canine heart is one of the models closest to human heart failure: the major advantage of this model is the availability of both *in vivo* and *in vitro* measurements of cardiac function (contractility and relaxation). It has been demonstrated using this model that the deterioration in the functional activity of the myocardial sarcoplasmic reticulum (Ca^{2+} transport and Ca^{2+}-ATPase activity) compared to controls closely correlates with the degree of deterioration of both diastolic and systolic function of the heart [88]. However, it should be mentioned that in contrast to the results obtained for small mammals and man (see below), Williams and coworkers [112] found no alteration in SERCA2a mRNA levels in paced dogs, although the animals clearly presented typical signs of heart failure and systolic and diastolic dysfunction.

In a small series of six human hearts with dilated cardiomyopathy, Movsesian and colleagues [82, 83] observed neither a reduced SR Ca^{2+} reuptake nor an alteration in SR Ca^{2+}-ATPase protein measured by immunoblotting. However, in larger series of human hearts, several other groups demonstrated a decrease in SERCA2a mRNA levels of about 30 to 40% [7, 47, 74, 105] as well as protein quantity [29, 47, 102] in human heart failure due to various cardiac diseases, including dilated cardiomyopathy, as compared to nonfailing myocardium. Moreover, the steady-state level of mRNA encoding PLN decreased in failing human myocardium like that of SERCA2a mRNA, suggesting that the expression of these two genes is coordinately regulated in failing human hearts [7, 33]. Also at the level of SR function, a significant reduction in Ca^{2+} reuptake by SR vesicles has been shown in failing hearts [47, 65], and measurements of intracellular Ca^{2+} transients revealed a decreased SR Ca^{2+} content in cardiomyocytes from failing human myocardium [16]. Interestingly, the levels of SERCA2a mRNA are positively correlated with cardiac functional indices [74] and the protein level of SERCA is correlated with the contractile properties of isolated papillary muscle strips [47]. A decreased SR Ca^{2+} reuptake rate secondary to a diminished SERCA expression could be regarded as the cause for the prolonged cytosolic Ca^{2+} transients with retarded diastolic Ca^{2+} decline, as measured *in vitro* in human failing myocardium using Ca^{2+} sensitive fluorescent indicators [17, 43]. It can be summarized, that there is a steadily increasing body of evidence for altered SR Ca^{2+} cycling as a hallmark of human heart failure. This can be the result of a decreased SERCA expression and/or an altered PLN-SERCA interaction as for example a reduced phosphorylation level of PLN in failing myocardium.

Ryanodine receptor: the Ca^{2+} release channel of the sarcoplasmic reticulum

Calcium release from the sarcoplasmic reticulum is mediated by a release channel referred to as the ryanodine receptor (RyR). It is constituted of 4 monomeric subunits of 564 kDa that form a tetrameric ion channel structure. Two different genes encode the cardiac isoform (RyR2) and the skeletal isoform (RyR1) (reviewed in [8, 89]). In rabbit heart, the expression of RyR2 is developmentally regulated, the level is about 9-fold higher in adult than in fetal myocardium [23]. Few studies have investigated RyR2 expression in human heart failure and these have provided controversial results: Brillantes and coworkers [22] observed a significant decrease in RyR expression only in hearts from patients with ischemic cardiomyopathy and no alterations in dilated cardiomyopathy compared to nonfailing myocardium. Without studying nonfailing hearts, Arai and coworkers [7] detected a negative correlation between RyR2 mRNA and the mRNA of atrial natriuretic factor (ANF) in failing myocardium independent of the underlying pathology but with broad interindividual variations: the more ANF mRNA was increased, the more RyR2 mRNA was decreased. Since ANF, which is not detectable in nonfailing ventricular myocardium, is regarded as a positively correlated marker for the degree of heart failure [33], this was interpreted as an indirect sign for a diminished RyR2 expression in failing hearts. Further investigation is needed for a better understanding of the RyR2 expression and its possible consequences in human heart failure.

Sarcolemmal Na^{+}/Ca^{2+} exchanger

In addition to the sarcoplasmic reticulum Ca^{2+}-ATPase implicated in cardiac relaxation, a second protein, the Na^{+}/Ca^{2+} exchanger located in the sarcolemma, contributes to the diastolic decline of intracellular Ca^{2+} concentration by extruding Ca^{2+} out of the myocytes. A single mRNA of 7 kb has been detected in the heart, with abundant levels also in the brain, suggesting the existence of only one cardiac isoform [60]. The mRNA sequence of this 120 kDa protein has been cloned in the dog [87] and man [60]. In rat models of aortic constriction or experimental myocardial infarction, it has been demonstrated that the activity of the Na^{+}/Ca^{2+} exchanger was depressed in sarcolemmal vesicular preparations, as compared to control animals [31, 44]. In contrast to what one would expect from the functional rat studies, Kent and coworkers [58] showed an increase in the expression of the Na^{+}/Ca^{2+} exchanger gene in an acute right ventricular pressure over-

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