Characteristics of Intracellular Ca^{2+} Handling Proteins in Heart Function in Health and Disease

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Abstract

In view of its ability to release and accumulate Ca^{2+}, the sarcoplasmic reticulum (SR) plays a major role in cardiomyocyte excitation–contraction coupling. Since Ca^{2+} is important in the functions of both mitochondria and nucleus, it appears that these organelles are involved in excitation–metabolism coupling and excitation–transcription coupling. This brief article discusses some of the characteristics of the SR, mitochondria, and nucleus with respect to their Ca^{2+} transport systems in cardiomyocytes and the role of Ca^{2+} in different coupling processes. In addition, the influence of fluctuations in the cytoplasmic Ca^{2+} concentration on cardiomyocyte contractile activity, metabolism, and gene expression in heart disease is presented. The potential of the SR and the mitochondria and nucleus as therapeutic targets for the treatment of heart disease is also highlighted.

Keywords

Heart · Excitation–contraction coupling · Excitation–metabolism coupling · Excitation–transcription coupling · Intracellular Ca^{2+} handling · Mitochondria · Nucleus · Sarcoplasmic reticulum · SERCA · Ryanodine receptors · Inositol trisphosphate receptor

21.1 Introduction

The precise control of intracellular Ca^{2+} cycling depends on the relationships among various channels and pumps that are involved in different membranous systems of cardiomyocytes. Accordingly, two significant aspects have been proposed: (a) structural coupling in which the transporters are organized within the dyad, linking the transverse tubule and sarcoplasmic reticulum and ensuring proximity of Ca^{2+} entry to sites of release and (b) functional coupling, where the fluxes across all membranes must be balanced such that, in the steady state, Ca^{2+} influx equals Ca^{2+} efflux on every beat [1]. Depolarization of the cardiomyocyte causes Ca^{2+} influx through the sarcolemmal membrane (SL) L-type Ca^{2+} channels (LTCCs), which subsequently elicits a greater release of Ca^{2+} upon activation of the sarcoplasmic reticulum (SR), ryanodine

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receptors (RyR), or Ca^{2+}-release channels. This increase in Ca^{2+} in the cytosol is rapidly dissipated by four mechanisms: (a) Ca^{2+} binding by proteins such as calmodulin and troponin [2], (b) Ca^{2+} efflux through SL or plasma membrane Ca^{2+}-pump ATPase (PMCA) and SL Na^{+}–Ca^{2+} exchanger (NCX), (c) storage in the SR by SR Ca^{2+}-pump ATPase (SERCA), and (d) Ca^{2+} buffering by intracellular organelles such as mitochondria and nucleus [3]. In this regard, the uptake of Ca^{2+} by the mitochondria occurs mainly through the mitochondrial Ca^{2+} uniporter (mCUP), while the nucleoplasmic reticulum serves as a Ca^{2+} storing site in the nucleus [4].

It is now well known that the process of cardiac contraction is initiated by the release of Ca^{2+} from the SR, whereas the process of relaxation is associated with Ca^{2+} uptake by the SR in cardiomyocytes. Furthermore, SR is also known to possess inositol trisphosphate receptors (IP_3R), the activation of which is considered to release Ca^{2+} in the cardiomyocyte [5, 6]. Fluctuations in the cytosolic levels of Ca^{2+} affect mitochondrial metabolism that may contribute to the pathogenesis of congestive heart failure (CHF) and ischemic heart disease (IHD) [5]. Cytosolic Ca^{2+} is also considered to regulate myocardial gene expression during subcellular remodeling in ischemia-reperfusion (I-R) injury, cardiac hypertrophy, and CHF [6, 7]. Accordingly, this chapter is intended to discuss the major characteristics of the Ca^{2+}-handling and regulatory proteins such as SR RyR, SR Ca^{2+}-pump ATPase, and IP_3R. Furthermore, the coupling of Ca^{2+} to the cardiac contraction–relaxation cycle, Ca^{2+} to mitochondrial metabolism, and Ca^{2+} to nuclear transcription is described following the excitation of cardiomyocytes. In addition, their role in the pathophysiology of different myocardial diseases will be briefly discussed, and the potential of the SR, mitochondria, and nucleus as therapeutic targets for heart disease will be highlighted.

21.2 Excitation–Contraction Coupling

The excitation–contraction (E–C) unit comprises synergistic lines of communication between the SL and SR [8]. Since the major functions of the SR are the release of Ca^{2+} for cardiac contraction upon excitation and uptake of Ca^{2+} for cardiac relaxation, it is planned to describe the characteristics as well as the role of SR Ca^{2+}-release channel, SR Ca^{2+}-pump ATPase and SR IP_3R (Fig. 21.1) in Ca^{2+} handling and regulation of the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) in cardiomyocytes.

21.2.1 SR Ca^{2+}-release Channel

The SR RyR or Ca^{2+}-release channel is a 564 kDa tetrameric protein consisting of at least four transmembrane segments and a large cytoplasmic domain. Three isoforms have been identified, namely, RyR1 and RyR3 in skeletal muscle and RyR2 in cardiac muscle; these isoforms share up to 60% sequence homology. RyR in cardiac muscle is organized in groups of approximately 20 receptors each. It has been suggested that the close approximation of these receptors is required

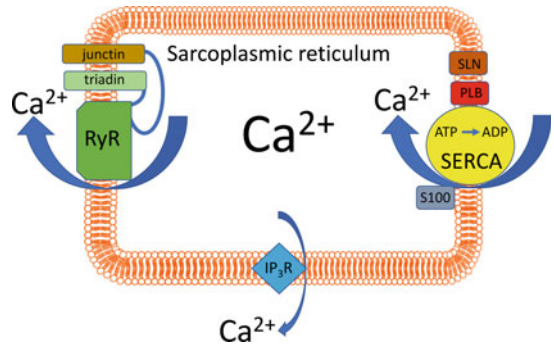

Fig. 21.1 Schematic representation of SR Ca^{2+}-handling proteins involved in the regulation of the intracellular concentration of Ca^{2+} in cardiomyocytes. *RyR* ryanodine receptor, *PLB* phospholamban, *SERCA* sarcoplasmic reticulum Ca^{2+}- pump ATPase, *IP_3R* inositol trisphoshate receptor

for orchestrated opening and closing of the Ca^{2+}-release channels [9]. Ca^{2+} has a biphasic effect on RyR; submicromolar Ca^{2+} concentrations activate RyR, whereas higher Ca^{2+} concentrations (>1 μM) inhibit the channel. RyR is inhibited by cytosolic Mg^{2+} but an increase in cytosolic Ca^{2+} relieves the Mg^{2+} inhibition [10]. RyR is also modulated by several cytosolic proteins; however, the most thoroughly studied modulators are calmodulin (CaM), calmodulin kinase (CaMKII), protein kinase A (PKA), and protein phosphatases. CaM binding to RyR is Ca^{2+} dependent, where Ca^{2+} binding increases the sensitivity of RyR to CaM. CaM binding inhibits the cardiac RyR in the presence of high concentration of Ca^{2+} (10 μM). Several kinases have been shown to affect the activity of cardiac RyR. These include protein kinase A, C, and G in addition to the CaM-activated CaMKII [6, 10]. The RyR phosphorylation is of great importance in the pathogenesis of heart failure and arrhythmias [11]. Hyperphosphorylation of RyR in heart failure leads to the dissociation of "calstabin", an accessory protein that stabilizes the closed conformation of the channel [12]. Phosphorylation of RyR cannot be interpreted in isolation from other SR proteins such as the Ca^{2+}-pump ATPase (SERCA) and phospholamban (PLB). PLB phosphorylation activates SERCA and increases the SR Ca^{2+} load, which then stimulates RyR [13]. While the overall effect of phosphorylation is stimulatory, protein phosphatases are inhibitory [10]. In addition to kinases and phosphatases, several other cytoplasmic proteins are considered to modulate the activity of cardiac RyR. These include the transmembrane proteins, junctin and triadin, and the cytosolic proteins, S100, sorcin, glutathione transferase as well as intracellular chloride ion channels, glycolytic enzymes, and the accessory protein, calstabin [14].

Mutations in cardiac RyR have been linked to the development of premature heartbeats. A "leaky" RyR promotes the buildup of Ca^{2+} in the cytosol and subsequently triggers the SL NCX to remove the excess Ca^{2+} in exchange for Na^{+}; this then depolarizes the cell membrane leading to extrasystolic depolarizations and premature beats. On the other hand, adrenergic stimulation leading to the phosphorylation of RyR can cause fatal arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT) in susceptible individuals. Susceptibility to CPVT was linked mainly to autosomal dominant mutations of RyR2; these mutations destabilize the channel or impair the binding to calstabin that stabilizes RyR [15, 16]. Recently, a mutation linked to the development of CPVT (R2474S) was found to produce "leaky" RyR in the heart and to the development of CPVT in transgenic mice. It has been shown that these RyRs could be stabilized by a novel compound, S107, for averting fatal arrhythmias [17]. Interestingly, the expression of RyR is reduced in heart failure due to the development of myocardial infarction (MI); this change is believed to be mediated through angiotensin receptors because it is inhibited by angiotensin-converting enzymes (ACE) inhibitors such as imidapril and in fact, close involvement of the SR RyR in the cellular remodeling process has been suggested [18]. In addition to suppressing cardiac hypertrophy, ACE inhibitor therapy has been shown to decrease the hyperadrenergic state associated with heart failure, resulting in a reduction of PKA activity, which abolishes the phosphorylation of RyR. Furthermore, ACE inhibitors have been reported to promote the interaction of RyR with the accessory protein calstabin, which stabilizes the channel and reduces the RyR leak [19].

21.2.2 SR Ca^{2+}-Pump ATPase

Most of the Ca^{2+} released from SR stores is rapidly taken up again via the SR Ca^{2+}-pump ATPase (SERCA), while the rest is extruded from the cell by the PMCA and SL NCX. The SERCA transports two Ca^{2+} ions for the hydrolysis of a single ATP molecule [19]. SERCA is organized into three interacting domains: the cytosolic nucleotide-binding domain, the phosphorylation domain containing Asp351, and the transmembrane translocation domain. The cytosolic and transmembrane domains consist of

10 transmembrane segments, which are connected by the stalk domain [20]. At least six isoforms of SERCA have been identified belonging to three different gene families, namely, SERCA1, 2, and 3; cardiomyocytes express the SERCA2a isoform [21]. The SERCA activity is regulated by SR-associated phospholamban (PLB). In this regard, the dephosphorylated form of PLB inhibits the pump by interacting with the enzyme phosphorylation site, which is needed for ATP binding, whereas phosphorylation of PLB by PKA or CaMK relieves this inhibition [22]. During adrenergic stimulation, PKA reduces the affinity of Ca^{2+}-pump ATPase for Ca^{2+} and thus enhances cardiac muscle relaxation. The adrenergic stimulation of the heart enhances the SERCA activity and such a regulatory action has been suggested to be a compensatory mechanism to improve cardiac performance.

Sarcolipin is another modulator of SERCA activity [23]. Sarcolipin is a shorter homologue (31 amino acids) of PLB, which inhibits SERCA. However, unlike PLB, the level of expression of sarcolipin determines its interaction with SERCA rather than its phosphorylation level. It has been found that sarcolipin modulates SERCA in a Ca^{2+}-dependent manner; at low Ca^{2+} levels, sarcolipin reduces its affinity for Ca^{2+} and thus inhibits its activity, whereas at high Ca^{2+} levels, it increases its affinity for Ca^{2+} by increasing the maximum turnover rate [24]. Sarcolipin and PLB both synergistically inhibit SERCA; coexpression of sarcolipin in HEK cells leads to a very strong inhibition of the SERCA activity, whereas a sarcolipin knockout leads to enhanced SERCA activity [25]. On the other hand, the S100 protein, which is abundantly expressed in the heart and skeletal muscle, is believed to stimulate Ca^{2+} uptake by SERCA2a through a direct protein–protein interaction [26].

In cardiac hypertrophy and heart failure, the levels of SERCA expression as well as the SERCA activity have been found to be reduced, leading to compromised SR function [27]. In fact, overexpression of SERCA can improve cardiac functional defects [28]. In heart failure, a reduction in the SERCA activity by about 50% was evident without any changes in the creatine kinase activity and mitochondrial functions [29]. While several studies have indicated a role for SERCA in heart function, heterozygous loss of function of the SERCA2a and 2b in Darier disease was not associated with any defects in cardiac performance [30, 31].

21.2.3 SR Inositol Trisphosphate Receptors

Although IP_3R plays a minor role in EC coupling compared to the RyR in ventricular cardiomyocytes [32, 33], the higher (3.5–10 fold) IP_3R expression in atrial myocytes vs. ventricular myocytes, is suggestive of a greater role of IP_3R in atrial contraction [34]. There are several isoforms of the IP_3R in both excitable and non-excitable tissues; the heart expresses the 300 KDa IP_3R2 isoform that co-assembles to form a tetrameric channel. The channel is predicted to have six transmembrane segments and a large regulatory cytoplasmic domain [35]. IP_3R1 is regulated by Ca^{2+} in a biphasic pattern similar to the RyR. In fact, it is activated by submicromolar concentration of Ca^{2+} ($<$300 nM) and inhibited by micromolar concentrations of Ca^{2+} ($>$1 μM). The cardiac IP_3R2 is resistant to inhibition by high Ca^{2+}, as it remains active in the presence of high Ca^{2+} concentration ($>$100 μM) [36, 37]. The IP_3R is modulated by cytosolic proteins such as calstabin, PKA, and CaM [38]. The most significant interaction of IP_3R is with CaM, because Ca^{2+}-free CaM inhibits the cardiac IP_3R2 in a Ca^{2+}-independent manner, indicating a permanent inhibition of these receptors by CaM in the absence of Ca^{2+} [39]. The development and progression of cardiac hypertrophy have been linked to increases in the phospholipase C-IP_3R expression levels leading to a stimulation of hypertrophic gene transcription. IP_3R2 has been shown to be upregulated, while, in contrast, a reduction in RyR2 expression and activity has been reported in heart failure [34]. In addition, the relative abundance of IP_3R in Purkinje fibers suggests its potential participation in ventricular arrhythmias

[40], however; the precise role of IP_3R in the generation of ventricular arrhythmias remains to be fully elucidated.

In addition to the role of SR in Ca^{2+}-handling in cardiomyocytes, other cellular structures can also be seen to participate in the process of excitation–contraction–relaxation cycle. It should be mentioned that induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) hold enormous potential in many fields of cardiovascular research. Overcoming many of the limitations of their embryonic counterparts, the application of iPSC-CMs ranges from facilitating the investigation of familial cardiac disease and pharmacological toxicity screening to personalized medicine and autologous cardiac cell therapies. The main factor preventing the full realization of this potential is the limited maturity of iPSC-CMs, which display a number of substantial differences in comparison to adult cardiomyocytes. E–C coupling, a fundamental property of cardiomyocytes, is often described in iPSC-CMs as being more analogous to neonatal than adult cardiomyocytes. With Ca^{2+}-handling linked, directly or indirectly to almost all other properties of cardiomyocytes, a solid understanding of this process will be crucial to fully realizing the potential of human iPSC-CMs [41].

21.3 Excitation–Metabolism Coupling

E–C coupling consumes a large amount of energy through myosin ATPase, the SL Na^+-K^+ ATPase, and SERCA activities [6]. On the other hand, the mitochondria are the main source of energy production. Mitochondria take up to 30% of the myocyte volume and are present in close proximity to the contractile machinery where energy is most required [42]. Indeed, $[Ca^{2+}]_i$ concentration in different cellular compartments is intimately linked to cell metabolism, because (a) ATP production requires low Ca^{2+}, (b) Ca^{2+} homeostatic systems consume ATP, and (c) Ca^{2+} signals in mitochondria stimulate ATP synthesis, being an essential part of excitation-metabolism (E-M) coupling [43]. The regulation of mitochondrial metabolism by Ca^{2+} is attributed to three key enzymes in the citric acid cycle, namely pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, which are activated by low concentrations of Ca^{2+} [44]; in addition, Ca^{2+} also activates the mitochondrial F_1/F_0 ATPase [45].

Mitochondria in cardiac muscles also possess the molecular components for efficient uptake and release of Ca^{2+}. In this regard, Ca^{2+} enters the mitochondrial membrane through the mitochondrial Ca^{2+} uniporter (mCUP), a low affinity (10–20 μM Ca^{2+}) and highly selective ion channel, which accumulates Ca^{2+} in the mitochondria using the potential difference across the mitochondrial membrane; the mCUP is Ca^{2+}-gated and requires calmodulin for its activation [46]. Although mitochondria are generally considered to serve as a Ca^{2+} sink, mitochondrial Ca^{2+} uptake is inhibited by high concentrations of cytosolic Ca^{2+} [47]. Ca^{2+} is extruded from mitochondria through the activity of mitochondrial Na^+-Ca^{2+} exchanger (mNCX), which is believed to possess a stoichiometry similar to that of the SL NCX of three Na^+ to one Ca^{2+} [48]. Since the Ca^{2+} extrusion mechanism is slower relative to the rate of Ca^{2+} entry, this leads to the accumulation of Ca^{2+} in the mitochondria [49]. Ca^{2+} and mitochondria may also be intimately linked to cardiac function as Ca^{2+} is central to cardiac excitation–contraction coupling and stimulates mitochondrial energy production to fuel contraction. It should be mentioned that under conditions of dysregulated Ca^{2+} cycling, mitochondrial Ca^{2+} overload activates cell death pathways [50]. Indeed, excessive accumulation of Ca^{2+} in the mitochondria may induce the translocation of cytochrome C as well as other pro-apoptotic factors [51]. Thus, taken together, the mitochondrial Ca^{2+} microdomain is where contraction, energy, and cardiomyocyte death converge [50, 52].

Studies have shown that increases in mitochondrial $[Ca^{2+}]$ occur simultaneously with the increase in cytosolic $[Ca^{2+}]$ in response to an increase in cardiomyocyte pacing or β-adrenergic stimulation [53, 54]. The rise of mitochondrial $[Ca^{2+}]$ was shown to be dependent

on the rise of cytosolic Ca^{2+} in the proximity of the SR, suggesting that mitochondria may also accumulate some of the Ca^{2+} released by the SR [55]. The ability of mitochondria to sense Ca^{2+} in a microdomain rather than the whole cytosol is predicted in modeling studies [56] and can explain the high threshold for Ca^{2+} uptake by the mCUP (1–3 μM). Indeed, localized Ca^{2+} sparks (synchronous coordinated activity of 30–100 RyR) elicit miniature mitochondrial matrix Ca^{2+} signals that last less than 500 ms [55]. The rapid buffering of cytosolic Ca^{2+} by mitochondria may be due to mCUP activity [57] or may be due to some other mechanism, which remains to be investigated.

E-M coupling is modulated by two messengers, ADP and NADH. The ADP production activates the mitochondrial F_1/F_0 ATPase to generate more ATP, which consequently leads to the reduction of inner membrane potential. This drop in potential activates NADH production (the second messenger) to supply electrons to compensate for the electron loss and maintain the inner mitochondrial membrane potential [58]. The other modulatory control of mitochondrial metabolism occurs through the mNCX, which is needed to maintain the intramitochondrial Ca^{2+} concentration. A study examining the influence of cytoplasmic Na^+ and mitochondrial potential on mNCX activity has confirmed the role of mNCX as a Ca^{2+} extrusion mechanism for the mitochondria [59]. In addition, it was demonstrated that mNCX is electrogenic and that depolarization of the mitochondrial membrane activates the mNCX to shuffle Ca^{2+} into the mitochondria rather than eliminating Ca^{2+}; this seems to be a potential protective mechanism against loss of mitochondrial potential that follows ATP depletion [58]. A third potential mechanism modulating E–M coupling is mediated by ATP-sensitive potassium (K_{ATP}) channels. These channels are activated by ADP and inhibited by ATP. It has been shown that K_{ATP} channels can be imported into the mitochondria and localize to the inner mitochondrial membrane following phosphorylation by protein kinase C. K_{ATP} are believed to have a protective effect against I-R-induced increase in cellular Ca^{2+} [60].

A rise in mitochondrial Ca^{2+} is associated with an increase in metabolism [61]. Cardiac pathologies, including CHF, IHD, or potential arrhythmias, lead to an increase in cytosolic Ca^{2+} and have a deleterious effect on mitochondrial metabolism [5]. In fact, a higher Na^+ concentration in the cytosol stimulates the mitochondrial NCX (mNCX), leading to reduced Ca^{2+} accumulation and reduced activity of mitochondrial function [49]. In cardiomyopathies, the reduced cytoplasmic Ca^{2+} negatively affects the mitochondrial Ca^{2+} transient, which is expected to inhibit mitochondrial function. Furthermore, a reduced rate of glycolysis, resulting in reduced availability of pyruvate, contributes to energy starvation in cardiomyopathy [62]. Thus, the mitochondria appear to be a therapeutic target in I-R injury and cardiomyopathies. It has been suggested that inhibition of the mNCX could be beneficial in cases of heart failure as well as during I-R injury.

In heart failure, the increase in cytosolic Na^+ activates the forward mode of mNCX to release mitochondrial Ca^{2+}. On the other hand, an increase in the cytosolic Ca^{2+} will trigger the reverse mode of mNCX and promote the accumulation of excess cytosolic Ca^{2+} in the mitochondria. As a consequence of its effect, the inhibition of mNCX has been shown to improve Ca^{2+} accumulation and mitochondrial energetics in isolated cardiomyocytes [62, 63]. Maintaining a delicate balance in mitochondrial Ca^{2+} content depends on the activities of mCUP and mNCX, where the balance between Ca^{2+} uptake and Ca^{2+} extrusion directly affects mitochondrial metabolism. Thus, inhibition of mNCX may be beneficial in preventing I-R injury and heart failure [5]. In addition, various interventions such as trimetazidine, ranolazine, dichloroacetate, carnitine palmitoyl transferase, and coenzyme Q10 have been proposed to prevent ischemic injury via mitochondrial modulation [63].

It is pointed out that current therapies for patients with stable systolic heart failure are largely limited to treatments that interfere with neurohormonal activation. Critical pathophysiological hallmarks of heart failure are an energetic deficit and oxidative stress, and both may be the result of mitochondrial dysfunction. This

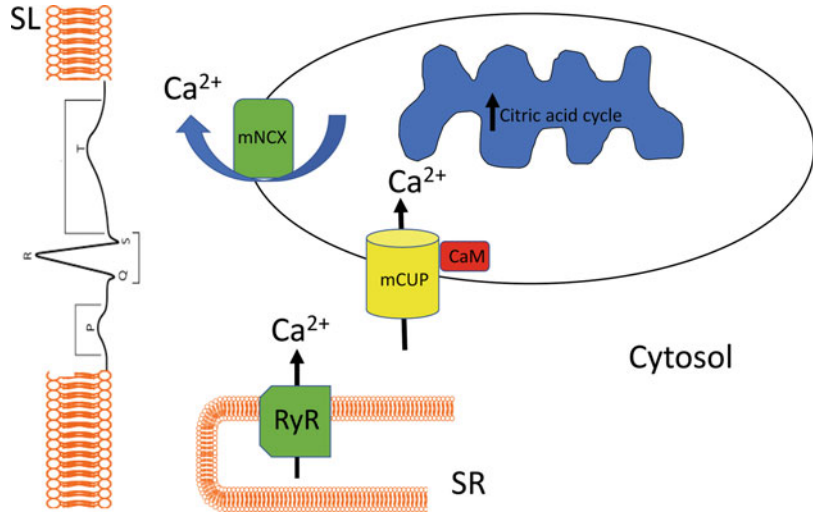

Fig. 21.2 Scheme depicting the role of mitochondria in the excitation-metabolism coupling in cardiomyocytes. *mCUP* mitochondrial Ca^{2+} uniporter, *CaM* calmodulin, *mNCX* mitochondrial Na^{+}-Ca^{2+} exchanger, *RyR* ryanodine receptor, *SL* Sarcolemma, *SR* sarcoplasmic reticulum

dysfunction is not only the result of a defect within mitochondria per se, but is in particular related to defects in intermediary metabolism. The regulatory interplay between excitation–contraction coupling and mitochondrial energetics, where defects of cytosolic Ca^{2+} and Na^{+} handling in failing hearts may play important roles [64]. The contribution and mechanisms of cardiomyocyte mitochondrial Ca^{2+} handling in E-C/E-M coupling as well as how mitochondrial Ca^{2+} regulates physiological mitochondrial and cellular functions in cardiac muscles in health and disease remain to be fully understood [65]. Figure 21.2 summarizes the major components of the E-M coupling process.

21.4 Excitation–Transcription Coupling

Ca^{2+} is a universal regulator of various cellular functions. In cardiomyocytes, Ca^{2+} is the central element of E–C coupling, but it also exerts a great impact on diverse signaling cascades and influences the regulation of gene expression, referred to as excitation–transcription (E–T) coupling. Disturbances in cellular Ca^{2+} handling and alterations in Ca^{2+}-dependent gene expression patterns are pivotal characteristics of failing cardiomyocytes, with several -E–T coupling pathways shown to be critically involved in structural and functional remodeling [66]. Electrical activity initiates a program of selective gene expression in excitable cells. Although such transcriptional activation is commonly attributed to depolarization-induced changes in intracellular Ca^{2+} [67], it should be noted that the specific and localized elevations of Ca^{2+} that are converted into changes in gene expression can be taken as long-term effects on the adaptation of the heart to a sustained stimulus [68].

The role of Ca^{2+} in controlling nuclear signaling has been established in some studies [69, 70]. In cardiomyocytes, nuclei are closely associated with SR; the nucleus of adult cardiomyocytes is reported to possess a nucleoplasmic reticulum, a nuclear Ca^{2+} store that is continuous with SR and the nuclear envelope [33]. The nucleoplasmic reticulum is loaded from cytosolic Ca^{2+} microdomains [71–74]. This organelle expresses functional IP_3R and RyR. There is evidence that the nucleus contains key components of the phosphoinositide-PLC signaling cascade, where the production of IP_3 has been speculated. It is suggested that the nucleus is able to control the effect of Ca^{2+} on gene expression, allowing nuclear Ca^{2+} to regulate cellular functions independently of the cytosolic Ca^{2+} increase [75]. Likewise, IP_3 can trigger the release of Ca^{2+} directly into the nucleoplasm, which may have an important impact on the excitation–

transcription process [75]. An increase in nuclear Ca^{2+} concentration is reported to control the Ca^{2+}-activated gene expression mediated by the cAMP response element [76]. Moreover, cytoplasmic and nuclear Ca^{2+} signals activate transcription through different pathways. Cytoplasmic Ca^{2+} signal activates transcription through the serum response element (SRE) transcription factor and does not require an increase in nuclear Ca^{2+} [77]. Similar to SR, the nucleoplasmic reticulum seems to possess counter-ion channels such as K^+ channels [78]; it is plausible that channels control the change in the potential across the nucleoplasm. This would be particularly important as the nucleoplasmic reticulum contains the voltage-gated R-type Ca^{2+} channels [78]. In addition, there is evidence that the nuclear membrane contains both NCX and NHE that may contribute to nuclear potential and cellular homeostasis.

CaMKII signaling regulates diverse cellular processes in a spatiotemporal manner including excitation–contraction and excitation–transcription coupling, mechanics and energetics in cardiac myocytes. Chronic activation of CaMKII results in cellular remodeling and ultimately arrhythmogenic alterations in Ca^{2+} handling, ion channels, cell-to-cell coupling, and metabolism [79]. Specific alterations in nuclear Ca^{2+} handling via altered excitation–transcription coupling contribute to the development and progression of heart failure. During cardiac remodeling, early changes of cardiomyocyte nuclei cause altered nuclear Ca^{2+} signaling implicated in hypertrophic gene program activation. Normalization of nuclear Ca^{2+} regulation may therefore be a novel therapeutic approach to prevent adverse cardiac remodeling [80].

The increase in nuclear Ca^{2+} signal has been closely associated with cardiac hypertrophy. The most well-characterized mechanism is via Ca^{2+}–CaM. This pathway is under the influence of Ca^{2+}–CaM–CaMK on histone deactylase (HDAC) or the effect of Ca^{2+}–CAM on the protein phosphatase calcineurin (CaN); the two ways may act in parallel, contributing to cardiac hypertrophy [51, 81, 82]. It has been shown that depolarization-mediated Ca^{2+} influx occurs through CaMKII to inhibit the HDAC5, thereby sustaining high activity of the cerebellar granule neuron maintained under myocyte enhancer factor 2 (MEF2) depolarizing conditions. In adult rabbit and mouse cardiomyocytes, phenylephrine and endothelin-1-induced nuclear export of HDAC5 depends not only on CaMK II but also on protein kinase D (PKD) [82]. The nuclear export required type II IP_3R, Ca^{2+} release from stores, CaM, HDAC5 phosphorylation but was completely insensitive to Ca^{2+} transients associated with both nuclear and cytosolic Ca^{2+} and PKC inhibition [51, 82]. HDAC class II is expressed in the heart and possesses a unique extension to bind MEF2, repressing its transcription activation; the relief of MEF2 repression by HDAC comes mainly through phosphorylation of HDAC by CaMK [83]. The latter argues for local control of Ca^{2+} release in the nuclear region, where local activation of nuclear IP_3R releases Ca^{2+} locally that activates CaMKII to phosphorylate HDAC and relieves transcription inhibition. This novel local Ca^{2+} signaling in excitation–transcription coupling is analogous to, but separate from, that involved in excitation–contraction coupling [51]. It is pointed out that MEF2 activation is strongly implicated in cardiac hypertrophy; this has been shown in transgenic animals overexpressing CaMKII and IV [83]. The link between MEF2 and CaMK is through HDAC [84].

Interestingly, acute activation of exchange protein activated by cyclic-AMP (Epac) has been shown to increase Ca^{2+} sparks and diastolic $[Ca^{2+}]_i$ but decrease systolic $[Ca^{2+}]_i$. Epac preferentially increases intranuclear Ca^{2+} concentration ($[Ca^{2+}]_n$) during both diastole and systole, correlating with the perinuclear expression pattern of Epac. Moreover, Epac activation induces HDAC5 nuclear export, with consequent activation of the prohypertrophic transcription factor MEF2. The cAMP-binding protein Epac modulates cardiac nuclear Ca^{2+} signaling by increasing $[Ca^{2+}]_n$ through PLC, IP_3R, and CaMKII activation and initiates a prohypertrophic program via HDAC5 nuclear export and subsequent activation of the transcription factor MEF2 [85].

Other transcription factors that are regulated by CaMK include activating protein (AP1), activating transcription factor (ATF-1), serum response factor (SRF), cyclic AMP response element (CREB), and the myocyte enhancing factor (MEF-2) [86]. While CREB can be phosphorylated by CaMKII, transgenic mice overexpressing CaMKII or CaMKIV that develop hypertrophy did not show an enhanced level of CREB phosphorylation [84, 87]. MEF2 activation is strongly implicated in hypertrophy; this has been shown in transgenic animals overexpressing CaMKII and IV [88]. The other mechanism by which Ca^{2+}–CaM controls transcription is through CaN, a Ca^{2+}-CaM binding phosphatase (500 times more sensitive to Ca^{2+} than CaM), allowing CaN to be more sensitive to small sustained Ca^{2+} transients [89]. CaN dephosphorylates NFAT, which leads to its translocation into the nucleus, where it binds to the transcription factor GATA4 to activate hypertrophic gene transcription [90]. In some studies, a sustained global rise in Ca^{2+} is needed to activate NFAT [91], whereas in others, Ca^{2+} oscillations were more efficient NFAT activators [92]. In neurons, the C-terminal fragment of LTCCs was found to be proteolytically cleaved as it translocated into the nucleus; this is then bound to a transcriptional regulator, Ca^{2+} channel associated transcription (CCAT) factor, leading to an increase in the length of neurites [93, 94]. The latter leads to an increase in some genes such as connexins, regulators of G-protein and catenin, while other proteins such as K^+ channel (Kcn3), NCX, myosin, NMDA receptor, serine-threonine kinase, and glucokinase are down-regulated.

While the role of this catalytic fragment in cardiomyocytes remains to be examined, the potential for developing specific inhibitors of nuclear Ca^{2+} signaling is unlimited. The control of such a mechanism could provide treatments for heart failure and cardiac hypertrophy as well as other conditions where Ca^{2+} oscillations affect gene expression and consequently the expression of signaling molecules that modulate Ca^{2+} cycling. It is now well recognized that CaMKII and a Ca-calmodulin-dependent phosphatase calcineurin are major Ca^{2+}-dependent mediators of transcriptional regulation. Moreover, these pathways contribute to changes in the gene expression of proteins involved in the HF phenotype, including some of the ion channels and Ca^{2+} transporters that are acutely involved in systolic dysfunction and arrhythmias [95]. In fact, the development of intracellular Ca^{2+} overload in the heart has been demonstrated to depress cardiac gene expression for SL Na^+–K^+ ATPase isoforms, SR RyR and SERCA2 proteins as well as α- and β-myosin [96] Fig. 21.3 summarizes the role of Ca^{2+} in E–T coupling in the cardiomyocyte.

21.5 Conclusions

From the aforementioned, it is evident that novel therapeutic interventions will continue to target ion channels; however, the focus may now also be on mitochondrial channels and exchangers in order to influence cellular metabolism and nuclear ion channels to control cell proliferation and growth. Targeting intracellular ion channels for the control of E-C, E-M and E-T coupling has emerged as an attractive new area of cardiovascular research that constitutes novel and exciting approaches for the treatment of IHD, cardiac hypertrophy, and heart failure.

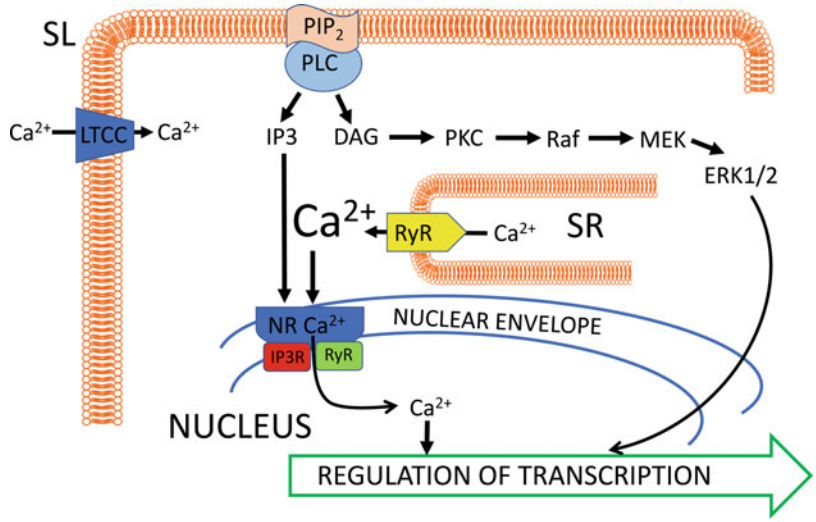

Fig. 21.3 Scheme showing the role of Ca^{2+} in excitation-transcription coupling in the cardiomyocyte. *NR* nucleoplasmic reticulum, *RyR* ryanodine receptor, *IP_3R* inositol trisphosphate receptor, *SL* sarcolemma, *LTCC* L-type Ca^{2+}-channel, *SR* sarcoplasmic reticulum, *PIP_2* phosphatidylinositol-bisphosphate, *PLC* phospholipase C, *DAG* diacylglycerol, *IP3* inositol trisphosphate, *PKC* protein kinase C, *Raf* Rapidly accelerated fibrosarcoma, *MEK* Mitogen-activated protein kinase kinase, *ERK1/2* extracellular signal-regulated kinase 1/2

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