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CaMKII delta subtypes: localization and function

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In this review we discuss the localization and function of the known subtypes of calcium/calmodulin dependent protein kinase II (CaMKII) and their role in cardiac physiology and pathophysiology. The CaMKII holoenzyme is comprised of multiple subunits that are encoded by four different genes called CaMKIIα, β, γ, and δ. While these four genes have a high degree of sequence homology, they are expressed in different tissues. CaMKIIα and β are expressed in neuronal tissue while γ and δ are present throughout the body, including in the heart. Both CaMKIIγ and δ are alternatively spliced in the heart to generate multiple subtypes. CaMKIIδ is the predominant cardiac isoform and is alternatively spliced in the heart to generate the CaMKIIδB subtype or the slightly less abundant δC subtype. The CaMKIIδB mRNA sequence contains a 33bp insert not present in δC that codes for an 11-amino acid nuclear localization sequence. This review focuses on the localization and function of the CaMKIIδ subtypes δB and δC and the role of these subtypes in arrhythmias, contractile dysfunction, gene transcription, and the regulation of Ca2+ handling.

Keywords: Ca2+/calmodulin-dependent protein kinase II, heart, splice variants, nuclear localization, transgenic mice

EXPRESSION AND LOCALIZATION

Calcium/calmodulin dependent protein kinase II (CaMKII) is a multimeric enzyme consisting of distinct subunits encoded by four different genes known as CaMKIIα, β, γ, and δ. These genes have a high degree of sequence homology but show differential tissue expression. CaMKIIα and β are predominantly expressed in neuronal tissue while γ and δ are present throughout the body, including the heart (Bennett et al., 1983; Tobimatsu and Fujisawa, 1989). CaMKIIδ is the predominant cardiac isoform and is alternatively spliced to generate multiple subtypes (Edman and Schulman, 1994).

Schwerer et al. (1993) were the first to demonstrate that there are different subtypes of CaMKIIδ expressed in various tissues. The authors reported four distinct proteins with differential expression patterns and named them CaMKIIδ1−4. CaMKIIδ2 and CaMKIIδ3 were shown to be identical except for the insertion of an 11-amino acid sequence in the variable domain of CaMKIIδ3, the more abundant of the two subtypes in the heart (Schwerer et al., 1993). Around the same time, Edman and Schulman (1994) identified the same CaMKIIδ subtypes in rat heart and characterized their catalytic activity and regulation by calcium-regulated calmodulin (Ca2+/CaM). They refer to the predominant cardiac subtypes as CaMKIIδB and CaMKIIδC (the convention that will be used in this review), which correspond to the δB and δC subtypes, respectively. The structure of these proteins is shown in Figure 1. CaMKIIδB and δC possess similar catalytic activity and sensitivity to Ca2+/CaM. Furthermore, both subtypes can undergo autophosphorylation and acquire a similar degree of Ca2+−independent or autonomous activity (Edman and Schulman, 1994). In the years that followed, seven additional splice variants of the CaMKIIδ gene, referred to as CaMKIIδ−11, were identified. Only one of these, CaMKIIδ9, is expressed in the adult heart (Figure 1; Mayer et al., 1994, 1995; Hoch et al., 1998, 1999).

The 11-amino acid insert in CaMKIIδB (38KRRKSSSVQMM) is also present in some splice variants of CaMKIIα and γ; this conservation suggests an important function (Schwerer et al., 1993). Srinivasan et al. (1994) showed that when constructs of CaMKIIδB are transfected into fibroblasts the expressed protein is localized to the nucleus. This is not the case for constructs of CaMKIIδC, implying that the additional amino acid sequence present in CaMKIIδB is responsible for nuclear localization (Srinivasan et al., 1994). A similar differential localization pattern was also observed when CaMKIIδ subtypes were expressed neonatal rat ventricular myocytes (NRVMs; Ramirez et al., 1997). Further studies showed that the 11-amino acid insert in CaMKIIδB can confer nuclear localization when inserted into the variable domain of CaMKIIα and that mutagenesis of the first two lysines in the insert abrogates the nuclear localization of these constructs. Thus it is widely accepted that the CaMKIIδB variable domain contains a nuclear localization signal (NLS).

CaMKII heteromultimerization is permissive in that the CaMKII holoenzyme can include subunits from multiple CaMKII genes and multiple splice variants of those genes (Bennett et al., 1983; Yamauchi et al., 1989). It seems likely that more than a single CaMKIIδB is present in a single CaMKIIδ multimer and accordingly the ratio of δB to δC in a multimer could regulate the localization of the holoenzyme. This has been demonstrated experimentally. When CaMKIIδB and δC are cotransfected into fibroblasts or NRVMs, the localization of the expressed protein can be shifted in accordance with the ratio of the expressed CaMKIIδB subtypes, i.e., highly expressed δB sequesters δB in the cytosol and blocks its nuclear localization (Srinivasan et al., 1994; Ramirez et al., 1997). The opposite is also true: high relative expression...
ASF/SF2 can be regulated by phosphorylation. Protein kinase A (PKA) mediates ASF/SF2 phosphorylation has been correlated with alternative splicing of CaMKIIδ in heart and brain (Gu et al., 2011). Additionally, regulation of ASF/SF2 by Protein phosphatase 1 γ (PP1γ) has been demonstrated to affect CaMKIIδ1 splicing (Huang et al., 2013). CaMKIIδγ expression is increased in models of isoproterenol-induced cardiac hypertrophy and thus regulation of CaMKIIδ splicing by PKA and PP1γ may be relevant in the context of chronic β-adrenergic stimulation (Li et al., 2011). The RNA binding proteins Fox 1 (RBFOX1) and 2 (RBFOX2) collaborate with ASF/SF2 to induce proper CaMKIIδ splicing (Han et al., 2011) and factors that regulate these proteins could also influence the expression of CaMKIIδ subtypes. Thus, CaMKIIδ splicing is a dynamic and regulated process. The role of this system in the heart has not been extensively explored but could be of major importance since regulation of CaMKIIδ splicing may account for altered subtype expression and CaMKIIδ signaling in physiological and pathophysiological settings.

**CaMKIIδγ TRANSGENIC MICE**

The differential localization and function of CaMKIIδ subtypes could be of considerable importance to understanding the role of this enzyme in normal physiology and disease states. Early studies demonstrated that expression of CaMKIIδB in NRVMs induced atrial natriuretic factor (ANF) expression and led to increased myofilament organization, both hallmarks of cardiac hypertrophy, while expression of CaMKIIδC did not (Ramirez et al., 1997). This finding suggested that nuclear CaMKIIδ localization is required to regulate gene expression. Consistent with this notion are data indicating that CaMKIIδB signaling activates several transcription factors including myocyte enhancer factor 2 (MEF2), GATA4, and heat shock factor 1 (HSF1; Little et al., 2009; Lu et al., 2010; Peng et al., 2010). The significance of the hypertrophic responses elicited by δB in vitro was explored further
by generation of CaMKIIδB transgenic (TG) mice (Zhang et al., 2002). These animals, which overexpress δB under the control of the cardiac-specific α-myosin heavy chain (α-MHC) promoter, demonstrate the enhanced expression of hypertrophic markers observed in NRVMs expressing CaMKIIδB. CaMKIIδB-TG animals develop hypertrophy and moderate cardiac dysfunction by 4 months of age. Thus, CaMKIIδB expression appears to be sufficient to induce cardiac hypertrophy. Surprisingly, despite the increased CaMKII activity in the CaMKIIδBTG mouse heart, phosphorylation of the canonical cardiac CaMKII substrate phospholamban (PLN) at its CaMKII site (Thr17) was not increased but rather was decreased relative to WT mice. PLN phosphorylation at the PKA site (Ser18) was similarly reduced. These data were related to increases in phosphatase activity (Zhang et al., 2002), but also implied that CaMKIIδB did not lead to robust phosphorylation of PLN. A subsequent paper that examined CaMKIIδB-TG animals at a younger age to avoid changes in phosphatase activity confirmed that phosphorylation of PLN and another cardiac CaMKII substrate, the cardiac ryanodine receptor (RyR2), was not increased by cardiac CaMKIIδB expression (Zhang et al., 2007). This finding is consistent with a predominantly nuclear localization and function of the δB subtype.

CaMKIIδB has also been suggested to regulate expression of the Na+/Ca2+ exchanger (NCX1) during the development of cardiac dysfunction following trans-aortic constriction (TAG; Lu et al., 2011). The conclusion that δB was the subtype involved in NCX1 regulation relied on the use of a constitutively active construct of CaMKIIδB in which a Thr to Asp mutation (T287D) simulates autophosphorylation. Interestingly, the authors found that this construct was excluded from the nucleus (Lu et al., 2010). This differs from the localization pattern described above (Srinivasan et al., 1994; Ramirez et al., 1997) and can be explained as a result of phosphorylation of Ser322 in the 11-amino acid insert of δB (Figure 1). The observation that mutation of Ser322 to Ala restores nuclear localization of constitutively active CaMKIIδB (Backs et al., 2006) confirms the role of this site in the cytosolic localization of the active construct. The possibility that phosphorylation of Ser322 might regulate CaMKIIδB localization in the intact heart has not been evaluated, but such a mechanism could contribute to the observation that CaMKIIδB is found outside the nucleus even in the absence of multimerization with δC (Mishra et al., 2011).

**CaMKIIδC Transgenic Mice**

CaMKIIδC transgenic mice have also been generated and demonstrate a strikingly different phenotype from mice that express CaMKIIδB. While cardiac dysfunction is relatively moderate and takes months to develop in CaMKIIδB-TG animals, mice expressing δC rapidly progress to heart failure and premature death (Zhang et al., 2003). By 6 weeks of age CaMKIIδC-TG animals display marked changes in cardiac morphology and by 12 weeks these animals display severe cardiac dysfunction and upregulation of hypertrophic genes.

**Ca2+ Handling and Arrhythmia**

Expression of the cardiac sarcoplasmic reticulum Ca2+-ATPase (SERCA) is diminished in δC-TG mice as occurs in other models of heart failure. Since SERCA regulates Ca2+ reuptake into the sarcoplasmic reticulum (SR), this decrease would diminish SR Ca2+ loading. On the other hand, the CaMKIIdc-TG mice show hyperphosphorylation of PLN at Thr17, which should improve SERCA function. In addition δC-TG animals display marked increases in phosphorylation of the RyR2, the channel through which Ca2+ exits the SR. Taken together, these changes would predict dysregulation of SR Ca2+ cycling and excitation–contraction coupling. This was substantiated in an accompanying paper that systematically analyzed and demonstrated dysregulation of cardiac Ca2+ handling in mice expressing δC (Maier et al., 2003). Specifically it was shown that SR Ca2+ stores were depleted in myocytes from these animals, explaining the observation that isolated myocytes displayed diminished twitch shortening amplitude. Furthermore, Maier et al. (2003) showed that the frequency and duration of Ca2+ sparks, or spontaneous intracellular Ca2+-release events, was markedly increased in myocytes from animals expressing δC. Hyperphosphorylation of RyR2 by CaMKIIdc was hypothesized to underly the enhanced leak of Ca2+ from the SR, and this was verified by the demonstration that acute inhibition of CaMKII in δC-TG myocytes rescues the altered Ca2+ handling (Maier et al., 2003). In other experiments, acute expression of δC in rabbit cardiomyocytes was shown to be sufficient to induce SR Ca2+ sparks and diminished SR Ca2+ loading (Kohlhaas et al., 2006). These findings imply that direct regulation of Ca2+ handling targets including RyR2 by CaMKIIdc can account for the dysregulation of Ca2+ and contractile function seen in myocytes from δC-TG animals (Figure 2).

Dysregulation of excitation–contraction coupling by CaMKII is thought to contribute to arrhythmogenesis in a variety of contexts, as supported by the increased incidence of arrhythmogenic events in CaMKIIdc-TG mice (Anderson et al., 1998; Wu et al., 2002; Wagner et al., 2006). Overexpression of CaMKIIdc not only induces more spontaneous arrhythmias but also enhances the susceptibility of mice to arrhythmic challenge by β-adrenergic stimulation, Sag et al. (2009) found that much of the proarrhythmic effects of β-adrenergic stimulation on SR Ca2+ leak were significantly inhibited by treatment of myocytes with KN-93, an inhibitor of CaMKII. Furthermore the SR Ca2+ leak induced by isoproterenol did not occur in myocytes from mice lacking CaMKIIδ. These findings collectively implicate SR Ca2+ leak as one of the key mechanisms in δC-mediated arrhythmias (Sag et al., 2009). The observation that hyperphosphorylation of RyR2 at the CaMKII site (Ser2814) contributes to arrhythmias and SR Ca2+ leak is supported by the finding that mutation of Ser2814 to Ala (S2814A) blocks the ability of CaMKII to induce Ca2+ sparks (van Oort et al., 2010). The autosomal dominant form of catecholaminergic polymorphic ventricular tachycardia (CPVT) can be caused by the RyR2 mutation R4496C and mice carrying this mutation are predisposed to arrhythmia and ventricular fibrillation. Enhanced CaMKIIδc expression and activity are implicated in the etiology of premature death in CPVT as expression of CaMKIIδc exacerbates the effects of the R4496C mutation (Dybkova et al., 2011). As mentioned earlier RyR2 Ser2814 phosphorylation is increased by expression of CaMKIIδc (but not by δB) in vivo (Zhang et al., 2007) and the effects of mutating this site (van Oort et al., 2010) emphasize the importance of RyR2 phosphorylation by CaMKII in SR Ca2+ leak and arrhythmia.
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CaMKIIδ subtypes: localization and function

FIGURE 2 | Localization and function of CaMKII δ subtypes in the adult cardiomyocyte. The circles labeled δC and δB represent CaMKII δ multimers that are composed primarily of δC and δB subunits, respectively. Documented phosphorylation events are indicated by dashed lines. CaMKII δC regulates Ca2⁺ homeostasis and currents involved in arrhythmogenesis through phosphorylation of Ca2⁺ handling proteins and channels. CaMKII δC can also affect gene transcription through direct and indirect mechanisms including phosphorylation of NFAT and HDAC (sequestering them in the cytosol), increases in p53, and increased nuclear import of NF-κB. The CaMKII δB subtype has little effect on phosphorylation of Ca2⁺ handling proteins but increases gene expression through HDAC phosphorylation and nuclear export and activation of HSF1 and GATA4. A putative mechanism for δB redistribution is depicted, showing δB exiting or being excluded from the nucleus due to phosphorylation at a site (Ser332) adjacent to its NLS.

Other targets besides those at the SR may contribute to the arrhythmogenic phenotype of CaMKIIδC mice. The cardiac sodium channel Nav1.5 is physically associated with CaMKIIδC based on coimmunoprecipitation of these proteins from CaMKIIδCTG animals and Nav1.5 is phosphorylated in mice expressing δC (Wagner et al., 2006). CaMKIIδC phosphorylates Nav1.5 at multiple sites and phosphorylation appears to elicit the loss-of-function changes in Nav1.5 gating that are observed in the context of CaMKIIδC expression in vitro (Ashpole et al., 2012; Koval et al., 2012). Incomplete inactivation of Nav1.5 generates a late Na⁺ current (I₉Na), which can prolong the duration of the action potential and contribute to arrhythmias. Additionally, increased I₉Na can lead to Na⁺-overloading of the cardiomyocyte, which contributes to diminished diastolic contractile performance (Maltsev et al., 1998). Late I₉Na is observed in CaMKIIδCTG mice and inhibition of this current ameliorates arrhythmia and diastolic dysfunction in these animals (Sossalla et al., 2011). Modulation of I₉Na therefore appears to contribute to the phenotype of CaMKIIδC mice with respect to arrhythmia development; additionally the CaMKIIδC subtype likely regulates the L-type Ca2⁺ channel (LTCC) and repolarizing potassium currents (Ito and IK₁; McCarron et al., 1992; Wagner et al., 2009). Thus, a multitude of mechanisms link CaMKIIδC to arrhythmogenesis.

CONTRACTILE DYSFUNCTION

Arrhythmias may contribute to the premature death of CaMKIIδCTG mice but there are also marked decreases in contractile function in these animals. Since alterations to cardiomyocyte Ca2⁺ handling are seen in relatively young CaMKIIδCTG mice and precede the development of heart failure, it is possible that dysregulated Ca2⁺ homeostasis (specifically SR Ca2⁺ leak) is an initiating event in δC-induced heart failure. Specifically, as a consequence of SR Ca2⁺ leak and SERCA downregulation, the SR Ca2⁺ load is diminished which would compromise contractile function. To determine whether diminished SR Ca2⁺ load is the primary causal event leading to contractile dysfunction and premature death in response to δC overexpression, we crossed the...
δC-TG mice with mice in which the SERCA regulatory protein PLN was deleted (PLN-KO). Deletion of PLN in the context of δC overexpression normalized SR Ca2+ levels and the contractile function of isolated myocytes was restored (Zhang et al., 2010). Remarkably the development of cardiac dysfunction in vivo was not rescued but instead was accelerated in the δC-TG/PLN-KO mice. In addition SR Ca2+ leak was enhanced. It was hypothesized that the increased SR Ca2+ load, in the context of RyR2 hyperphosphorylation, precipitated greater Ca2+ leak and further suggested that the accelerated development of cardiac dysfunction was due to mitochondrial Ca2+ overloading (Zhang et al., 2010). These observations and their interpretation places central importance on the Ca2+ leak elicited by δC-mediated phosphorylation of RyR2 in the development of heart failure. Further support for this hypothesis comes from the finding that CaMKII δ knockout mice have attenuated contractile dysfunction in response to pressure overload induced by TAC and myocytes from these animals show diminished SR Ca2+ leak in response to TAC (Ling et al., 2009). Additionally, mice expressing the RyR2 S2814A mutation are protected from the development of heart failure in response to pressure overload (Respress et al., 2012) consistent with a critical role for CaMKII-mediated RyR2 phosphorylation. We recently crossed CaMKIIδc mice with those expressing RyR2 S2814A; if the hypothesis is correct these mice will show diminished SR Ca2+ leak and improved contractile function when compared to CaMKIIδc-TG mice.

Another approach used to determine the role of RyR2 phosphorylation and SR Ca2+ leak in the phenotype of CaMKIIδc-TG mice was to cross the CaMKIIδc-TG mice with mice expressing SR-targeted autocamtide-2-related inhibitory peptide (SR-AIP; Huke et al., 2011). AIP simulates the regulatory domain of CaMKII and inhibits the kinase, and SR-AIP mice have been shown to display diminished phosphorylation of CaMKII substrates at the SR (Ji et al., 2003). A reduction in the extent of PLN and RyR2 hyperphosphorylation observed in CaMKIIδc-TG mice was conferred by SR-AIP. There were associated changes in Ca2+ handling that indicated a modest improvement in SR Ca2+ leak. Despite the salutary effects of SR-AIP in cells from δC-TG mice, in vivo cardiac function was not improved. One possible explanation for these findings is that the degree of inhibition of RyR2 phosphorylation conferred by SR-AIP was insufficient to prevent the effects of CaMKIIδc overexpression. Alternatively, while δC-mediated phosphorylation of targets at the SR including RyR2 and PLN is of considerable consequence, targets of CaMKII elsewhere in the cell may also contribute to the pathogenesis of cardiac dysfunction induced by CaMKIIδc.

Mitochondrial Ca2+ is elevated in mice overexpressing δC in the context of intact SR Ca2+ load (Zhang et al., 2010) and increases in mitochondrial Ca2+ are known to induce opening of the mitochondrial permeability transition pore (MPTP) and cell death (Halestrap and Davidson, 1990). Considering the central importance of mitochondria in the regulation of cell death and of cell death in the development of heart failure (Wencker et al., 2003), any pathway by which CaMKII δc induces mitochondrial Ca2+ overloading and subsequent loss of mitochondrial integrity would be predicted to contribute to the development of contractile dysfunction and heart failure. To test the role of mitochondrial dysregulation in the cardiomyopathy that develops in δC-TG animals, CaMKIIδc-TG mice were crossed with mice lacking expression of cyclophilin D, a mitochondrial protein required for the formation of the MPTP. The ability of high Ca2+ to induce swelling of isolated mitochondria, an index of MPTP opening, was impaired in the CaMKIIδc-TG mice lacking cyclophilin D, but development of dilated cardiomyopathy and premature death of these mice was not diminished. Indeed these responses were exacerbated when compared to δC-TG mice with intact cyclophilin D expression. The authors suggest that cyclophilin D may actually play a beneficial role in stress responses, as they observed that TAC-induced heart failure development was also made more severe by genetic deletion of cyclophilin D (Elrod et al., 2010). However, CaMKIIδc is found at mitochondria and a recent seminal study by Joiner et al. (2012) identified the mitochondrial Ca2+ uniporter (MCU) as a potential target of CaMKII (Mishra et al., 2011; Joiner et al., 2012). While phosphorylation of the MCU by CaMKII was not shown to occur in vivo, a CaMKII-dependent change in the function of the MCU was evidenced by data demonstrating that a CaMKII inhibitory peptide targeted to the mitochondria diminished mitochondrial Ca2+ uptake and inhibited apoptosis in mice subjected to myocardial infarction and I/R injury.

**CaMKIIδ SUBTYPES IN GENE TRANSCRIPTION**

The discussion above, and indeed most of the literature, considers the role of CaMKIIδ-mediated phosphorylation and regulation of Ca2+ handling proteins and ion channels. Chronic elevations in CaMKIIδ expression and activity are observed in humans with heart failure (Hoch et al., 1999) and these long-term changes are likely to elicit altered gene expression. As discussed earlier, CaMKIIδ induces the expression of hypertrophic genes in myocytes and transgenic mice, consistent with its primarily nuclear localization (Ramirez et al., 1997; Zhang et al., 2002). Other work showed that the CaMKIIδ subtype is required for GATA-4 binding to the B cell lymphoma 2 (Bcl-2) promoter and subsequent gene expression (Little et al., 2009). Furthermore, CaMKIIδ was shown to phosphorylate the transcription factor HSF1 thereby increasing its transcriptional activity (Peng et al., 2010). Taken together, these observations imply that it is the CaMKIIδ subtype that regulates gene expression as a result of its actions in the nucleus.

It is not necessarily the case, however, that gene regulation requires CaMKIIδ to be localized to the nuclear compartment. Despite its primarily cytosolic localization, CaMKIIδC overexpressed in mouse heart increased phosphorylation of histone deacetylase 4 (HDAC4), resulting in activation of the transcription factor MEF2 (Zhang et al., 2007). CaMKIIδC has also been demonstrated to regulate nuclear localization of nuclear factor of activated T cells (NFATs) in NRVM. The ability of CaMKIIδC to decrease nuclear NFAT was blocked by coexpression of a dominant-negative construct of CaMKIIδC and was shown to be elicited by phosphorylation and inhibition of the Ca2+/CaM dependent phosphatase calcineurin (Cn; MacDonnell et al., 2009), presumably in the cytosol. Alteration of Ca2+ homeostasis by cytosolic CaMKIIδc expression may indirectly affect gene expression and additionally the constitutively active CaMKIIδc utilized in the studies discussed
above (Lu et al., 2011) is cytosolic and yet regulates expression of NCX1.

Regulation of gene expression by CaMKIIδ has been demonstrated to promote cardiomyocyte survival while the opposite is true for CaMKIIδC. CaMKIIδB was shown to protect cardiomyocytes from doxorubicin-induced cell death via transcriptional upregulation of Bcl-2 (Little et al., 2009). Along similar lines, CaMKIIδB contributes to cardioprotection from H2O2 by increasing inducible heat shock protein 70 (iHSP70) expression (Peng et al., 2010). Conversely, CaMKIIδC activation is implicated in cell death elicited by a variety of stimuli (Zhu et al., 2007). It has been suggested that CaMKIIδC (but not δB) upregulates the proapoptotic transcription factor p53 (Toko et al., 2010), and recent work from our laboratory demonstrates that CaMKIIδC expression in NRVMs activates the proinflammatory transcription factor nuclear factor κB (NF-κB; Ling et al., 2013). We demonstrated that CaMKIIδC increased phosphorylation of IKK (IKK) and since IKK activation can also upregulate p53 (Jia et al., 2013), this pathway may contribute to the proapoptotic response reported by Toko et al. (2010).

**FUTURE DIRECTIONS**

There is compelling evidence that the CaMKIIδ subtypes differentially regulate cardiomyocyte Ca2+ handling and survival in vitro. Whether this occurs in vivo under physiological or pathophysiological conditions, and whether δB and δC subserve different functions based on their localization or selective activation, remains to be determined.

It seems likely that the relative levels of endogenous δB and δC determine localization and could therefore impact CaMKII signaling. Hypothetically, a selective increase in CaMKIIδB would result in accumulation of cytosolic CaMKIIδ and depletion of nuclear CaMKIIδ while a selective increase in CaMKIIδB would have the opposite effect. CaMKIIδ redistribution in this manner may contribute to the phenotype of mice that overexpress δB and δC and importantly there are changes in the relative expression of δB and δC in models of heart failure and I/R injury. In both models δC expression is enhanced relative to that of δB (Zhang et al., 2003; Peng et al., 2010). It is not known how this occurs but it is of interest to postulate that in heart failure and during I/R regulation of CaMKIIδ splicing is altered. ASF/SF2 and RBFOX1/2 regulate the splicing of the CaMKIIδ gene and thus expression of δB and δC, but whether changes in splicing occur in and contribute to the development of heart failure or I/R injury remains to be determined. It is likely that the increased δC expression observed in these models is pathogenic.

While CaMKIIδB contains an NLS, this subtype is not completely sequestered in the nucleus (Mishra et al., 2011). As mentioned previously the NLS within the variable domain of δB can be regulated by phosphorylation, which prevents nuclear localization. This type of regulation could be of considerable importance since the nuclear localization of δB appears to correlate with enhanced expression of protective genes and cell survival while cytosolic localization does not (Little et al., 2009; Peng et al., 2010; Lu et al., 2011).

Of additional interest is the neglected CaMKIIδB. The pioneering work of (Hoch et al., 1998; Mayer et al., 1995) identified δB as one of the three subtypes of CaMKIIδ in the adult heart and showed that it is expressed at similar levels to those of CaMKIIδB, δB contains a sequence (328EPQTTVIHNPDGK) not present in δB or δC and thus may possess unique properties that merit further investigation, as the function and localization of δB in vivo has not been explored. Along similar lines, CaMKIIδA expression is increased in a model of cardiac hypertrophy (Li et al., 2011), but the possibility that this splice variant is upregulated in and contributes to cardiovascular disease has not been investigated.

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**REFERENCES**


Kohlhaas, M., Zhang, T., Seidler, T., Zibrova, D., Dybkova, N., Stein, A., et al. (2006). Increased sarcoplasmic reticulum calcium leak but unaltered contractility by acute CaMKII overexpression in isolated rabbit cardiac myocytes. Circ. Res. 98, 235–244. doi: 10.1161/01.RES.0000200739.90811.9f


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