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Protein Kinase Signaling at the Crossroads of Myocyte Life and Death in Ischemic Heart Disease

Ronald J. Vagnozzi, Nicholas E. Hoffman, John W. Elrod, Muniswamy Madesh, and Thomas Force

Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3026, USA

Abstract

Myocardial ischemia results in death of cardiac myocytes via tightly-regulated and interconnected signaling pathways. Protein kinases play crucial roles in this regulation and are highly amenable to therapeutic intervention, making targeted inhibition an attractive strategy for ischemic heart disease. Recent studies have uncovered numerous kinases that participate in the cardiomyocyte response to ischemic injury, thus potentiating the development of new therapeutics. Moreover, many kinase signaling pathways converge at the mitochondria, a key participant in both cardiomyocyte physiology and the pathogenesis of ischemic heart disease. Herein we highlight kinase pathways regulating three major drivers of cell death: mitochondrial permeability transition pore opening (mPTP), programmed necrosis and Ca^{2+} overload-induced mitochondrial dysfunction. Inhibition of each of these kinase pathways has been proposed as a means to limit cardiomyocyte death from ischemia/reperfusion (I/R) injury.

Introduction

Heart failure has reached epidemic levels worldwide and is associated with profound morbidity and mortality. Although the etiology of heart failure is strikingly complex, in many instances it develops progressively in patients who have suffered a myocardial infarction (MI), resulting in tissue ischemia and subsequent death of cardiac myocytes. Overall patient prognosis post-MI, including whether the patient will ultimately progress to heart failure, is largely dependent on the extent of myocyte loss during this initial ischemic insult and the gradual cell dropout associated with the progression of disease. Despite an overwhelming medical need, therapies that reduce myocyte death post-MI have remained elusive, and drug development in this area has largely stagnated. Thus the field is very much in need of novel targets and more effective approaches to therapeutics.

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Conflict of Interest

The authors report no conflicts of interest associated with this work.

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Modulation of protein kinase signaling has gained increased attention as a means to limit cardiomyocyte death. Myocyte loss due to ischemia/reperfusion (I/R) occurs by both necrosis and apoptosis, and recent reports have suggested phosphorylation events via downstream kinases may directly modulate these cell death pathways. These mechanisms, at least in part, have also been shown to modulate mitochondrial function or mitochondria-mediated cell death. Growing evidence recognizes the mitochondria as a major effector of cardiomyocyte death and dysfunction in ischemic heart disease. Consequently, kinases acting at “nodal points” to regulate multiple forms of myocyte death, including mitochondria-mediated death, may represent particularly promising avenues for therapeutic intervention.

Mitochondrial Permeability Transition: A Pivotal Event in Myocyte Life or Death

Maintenance of mitochondrial membrane integrity, particularly the regulated permeability of the inner mitochondrial membrane (IMM), is essential for ATP generation and metabolic and energetic homeostasis in the cell. As such, mitochondrial permeability transition (mPT), a dynamic event in which solutes up to 1.5 kD in mass freely cross the IMM [1], can have profound effects on cellular bioenergetics as well as cell survival. The mitochondrial permeability transition pore (mPTP) is the key mechanistic effector of mPT. The structure and composition of the mPTP remains largely unknown, with cyclophilin D (CypD) being the only established molecular component [2–4]. Historical models of the pore included adenine nucleotide translocase (ANT) transporters and VDAC [5], both of which have not held up to genetic scrutiny as bona fide components of the pore itself, although they do regulate mPT. The mitochondrial phosphate carrier (PiC) can also regulate mPTP opening, but it is not yet clear whether PiC is an actual pore component.

Under physiological conditions, it has been suggested that mPTP opening regulates mitochondrial Ca^{2+} extrusion [6–8], and this may also facilitate proper metabolic function of the mitochondria [9]. However, considerably more focus has been placed on the pathological roles of the mPTP in several forms of cell death. The mPTP is highly responsive to cellular stresses, including changes in intracellular pH, mitochondrial membrane potential, mitochondrial matrix Ca^{2+} levels, or reactive oxygen species (ROS). Ultimately, the consequences of prolonged mPTP opening include impaired ATP production, ROS elevation (a phenomenon known as ROS-induced ROS release) and the swelling and lysis of mitochondria, leading to cell death by necrosis. In cardiac systems, the mPTP has been implicated in the pathophysiology of ischemic injury for more than two decades [10]. Moreover, numerous studies have established the mPTP as a major effector of reperfusion injury during pathological stress [11–13], and a potential target for drug development [14,15]. Since then, considerable efforts have broadened our knowledge on the mechanisms of mPTP activation, including modulation by protein kinase signaling, and the resulting cellular consequences.

Kinase Regulation of mPTP: The PI3K-Akt-GSK3 axis

Although several signaling pathways have been implicated in mPT, the precise mechanisms by which mPTP opening is regulated remain undefined. One principal pathway implicated in mPTP regulation is the PI3K-Akt-GSK3 axis. Akt, or protein kinase B (PKB), is a serine-threonine kinase and a master regulator of cellular responses such as survival, proliferation, nutrient sensing/utilization and growth. In the heart, the literature on Akt is extensive [16] and has largely established Akt as a key pro-survival kinase in normal cardiac homeostasis and in response to injury. Classically, Akt activation via upstream phosphatidylinositide 3-kinases (PI3Ks) promotes survival via inhibition of pro-apoptotic Bcl-2 family proteins Bax and Bad, limiting mitochondrial outer membrane (OMM) permeabilization and thereby blocking release of cytochrome *c* and caspase-mediated apoptosis.

The glycogen synthase kinase 3 (GSK-3) serine/threonine kinases, GSK-3 α and GSK-3 β , are key downstream targets of Akt which are active when dephosphorylated. Phosphorylation of downstream substrates by GSK-3 confers deactivation; thus GSK-3 phosphorylation by Akt or other upstream mediators results in inhibition of GSK-3-regulated targets. Inactivation of GSK-3 β by Akt reduces mitochondrial Bax recruitment [17] as well as mPTP opening [18]. Later studies showed that GSK-3 β localizes to mitochondria following inactivation (phosphorylation) and resides in the same fraction as ANT and VDAC [18–20]. While these studies did not prove direct association with the mPTP, they support a role for GSK-3 β as a regulator of pore activity. Whether GSK-3 β acts directly on the mPTP, or mediates mPTP indirectly via intermediate substrates is not yet known. In a myocardial postconditioning model utilizing phospho-dead GSK-3 β mutant mice [21], cytoprotective postconditioning was shown to require GSK-3 β inactivation. However, mitochondria isolated from the phospho-dead mutant showed no alteration in Ca²⁺ retention capacity, suggesting that modulation of the mPTP by GSK-3 β is most likely independent of CypD. In other studies, GSK-3 β has been shown to directly phosphorylate CypD, albeit using recombinant protein systems [22], but the effect of this phosphorylation on CypD function, or its occurrence in vivo, has not been determined [9]. GSK-3 β also has been reported to phosphorylate Bax, inducing its mitochondrial translocation [23]. In addition to its role in apoptosis, Bax itself has recently been implicated as a mediator of mitochondrial IMM permeabilization and necrosis [24,25], representing another layer of GSK-3 β regulation. These and other studies make the Akt-GSK-3 β signaling axis a nexus of pro-survival signaling and one major point of convergence between apoptotic and necrotic death regulation. Thus inhibition of GSK-3 β has been frequently proposed as a prominent target for cardioprotection.

In contrast to GSK-3 β , the role of GSK-3 α in cardiomyocyte death is largely unexplored. Recent data from the Force lab suggested that GSK-3 α plays a critical role in limiting ischemic injury. These studies showed that loss of GSK-3 α increased cardiomyocyte apoptosis post-MI or in response to hypoxia, in part by promoting mitochondrial Bax recruitment and subsequent cytochrome *c* release [26]. Thus regulation of cardiomyocyte death by GSK-3s in I/R appears to be highly isoform-specific, in that GSK-3 α promotes survival, whereas GSK-3 β promotes both necrosis and apoptosis. Thus the development of

isoform-specific inhibitors remains a crucial obstacle in how to therapeutically limit myocyte loss via blocking GSK-3 β , or several of the other kinases we next discuss.

Additional Targets of Akt-mediated Regulation of mPTP

Hexokinase

Hexokinase phosphorylates glucose to form glucose-6-phosphate, a limiting substrate in glycolysis. Hexokinases can associate physically to the outer membrane of mitochondria (OMM) through interaction with VDAC and presumably this interaction may position hexokinase at ATP microdomains and thereby act as a sensor or switch to maintain energy homeostasis (ATP is a necessary substrate for hexokinase activity). Hexokinase has been shown to interact with VDAC, and this has been proposed as an inhibitor of permeability transition [27,28]. Exactly how hexokinase detachment from mitochondria can modulate mPTP opening remains unclear. One possibility may be via an alteration in cellular redox status, as isolated mitochondria studies suggest that the VDAC-hexokinase interaction is important in ADP recycling, suppression of ROS production, and maintenance of mitochondrial membrane potential [29,30]. Of note, phosphorylation of hexokinase by Akt at the mitochondria has been proposed to regulate this interaction. Indeed, mitochondrial dysfunction and Ca²⁺ handling following oxidative stress were ameliorated by Akt activation, via a mechanism that depended on hexokinase association with mitochondria. However, further confusing the true nature of hexokinase in mPTP control is a report using genetic manipulation that revealed hexokinases' role in mPTP-mediated cell death is independent of VDAC but yet requires CypD and/or ANT [31]. Taken together, hexokinase activity downstream of Akt seems to play an important role in modulation of mPTP, but further studies are required to fully define the mechanisms of this effect.

p53

A recent study by Vaseva *et. al.* has implicated p53 as another key mediator of mPTP. The authors reported that p53 can induce necrosis via interaction with CypD, specifically in the setting of oxidant stress [32]. Akt is also known to regulate p53 via activation of murine double minute 2 (MDM2), an E3 ubiquitin ligase. In the cardiomyocyte, p53 is induced in response to stresses such as ischemia and exerts powerful pro-death effects [33]. Loss of MDM2 increases p53 expression and I/R injury in the mouse heart [34], while MDM2 over-expressing mice show protection in this model. MDM2 phosphorylation by Akt increases MDM2 activity, nuclear import, and protein stability, which enhances MDM2 activity to confer protection from apoptosis [35]. From this it is tempting to speculate that Akt-mediated inhibition of p53 via MDM2 could also impart protection from mPTP and necrosis [36]. However, the authors found no alteration in Ca²⁺-dependent mPTP opening by p53, which is arguably the most fundamental regulator of permeability transition. This and other inconsistencies [37] suggest that p53-mediated cell death may occur via an indirect effect on mPTP. Indeed, numerous studies have shown p53 to activate and oligomerize Bcl2 proteins to permeabilize the OMM, including work from the same lab [38]. Thus many key questions remain as to the precise mechanisms of p53 mediation of mPTP, and potential application for cardiovascular therapy.

Programmed Necrosis/Necroptosis: A Merged Lane to Cell Death

Cell death by necrosis is a fundamental pathological event. In the ischemic heart, the ultrastructural features of necrosis, such as cellular swelling, membrane damage and cell rupture, were demonstrated more than forty years ago. Since then numerous studies have supported the concept that necrosis predominates as the form of cellular injury in myocardial infarction [39]. In response to ischemia, a significant portion of cardiac cells (notably myocytes but also cardiac fibroblasts and cells of the vasculature) die by necrosis due to overwhelming cellular damage. Loss of oxygen and nutrients in cardiac myocytes leads to rapid depletion of cellular ATP, resulting in mitochondrial dysfunction, decreased intracellular pH, and energetic imbalance as cells shift from aerobic to anaerobic respiration. Compromised myocytes rapidly lyse, releasing cytoplasmic and mitochondrial proteins, free radical reactive oxygen species (ROS), and other pro-inflammatory and cytotoxic factors which subsequently contribute to further myocyte loss [5] [40] [41].

Classically regarded as a passive consequence of tissue damage, recent studies have revealed a highly regulated system of inducible, “programmed” necrosis via signaling through the pro-inflammatory death receptors, including: TNF α receptor, TNF-related apoptosis-inducing ligand (TRAIL), Fas, or toll-like receptors (in cardiomyocytes, predominantly TLR2 and TLR4). Upon death receptor activation, the fate of the stimulated cell is dictated by the regulated assembly and disassembly of a macromolecular protein complex termed the “necrosome” or “necroptosome” [42], which can include more than a dozen different proteins [43]. Of note, mPTP has also been put forth as a major component of programmed necrosis, although it is not yet clear whether death receptor signaling is a direct contributor to mPTP. Nevertheless, the conventional wisdom that necrosis and apoptosis represent distinct and disconnected cellular phenomena has largely transformed into a more dynamic view of cell death, containing aspects of both and subject to convergent regulatory pathways.

Kinase Regulation of Programmed Necrosis: The Receptor Interacting Protein (RIP) Pathway

Signaling for programmed necrosis converges on the receptor interacting protein 1 and 3 (RIP1, RIP3) kinases. RIP1 was first identified from a yeast two-hybrid screen due to its ability to bind the Fas death receptor via a C-terminal death domain [44]. Later studies showed RIP1 also binds the death receptors Tumor necrosis factor receptor 1 (TNFR1) and Tumor necrosis-mediated apoptosis-inducing ligand receptors 1 (TRAILR1) and 2 (TRAILR2) [42]. RIP1 also interacts with its partner kinase, RIP3, through a RIP homotypic interaction motif (RHIM) [45]. Both RIP1 and RIP3 have an N-terminal serine/threonine protein kinase domain required for activation of various RIP1/3 interacting partners [45]. Subsequent RIP-mediated signaling is therefore driven by the selective recruitment of these RIP1/RIP3 interacting partners (the necrosome), initially at the activated death receptor.

RIP1/3 signaling leads to increased ROS production and subsequent cytotoxicity [43]. Moreover, RIP1 kinase activity induces necrosis by activating Fas/TNF-R/TRAIL-R signaling [46–49]. This induction of necrosis is highly dependent on the interaction partners

recruited to RIP1/3. In Fas-initiated necrotic cell death, RIP1 binds to the death-inducing signaling complex (DISC) of Fas [46]. In TNF- α initiated cell death RIP1 forms a superoxide-producing complex by interacting with the NAD(P)H oxidase NOX1, its adaptor protein NOXO1, TNFR1-associated death domain protein (TRADD) and Rac1 [50]. RIP1 has also been implicated in TLR stimulation and DNA damage-induced necrosis [51–53].

Regulation of RIP1 and RIP3 via post-translational modification also plays a role in the cellular decision to undergo apoptosis (vs. necrosis). Ubiquitination and acetylation both are suggested to regulate RIP-mediated signaling. Non-ubiquitinated RIP1 binds second mitochondria-derived activator of caspase (Smac), which activates RIP1 and facilitates the formation of a complex containing RIP1, RIP3, TRADD, Fas-associated death domain protein (FADD), and caspase-8 (termed complex I). In this complex RIP1 and RIP3 are cleaved by activated caspase 8 [54] thus suppressing necrosis in favor of caspase 8-mediated apoptosis [55,56]. Upon deactivation of caspase-8 by caspase inhibitors, the degradation of RIP1 and RIP3 is inhibited, allowing for phosphorylation of RIP1 and RIP3 to occur. Phosphorylated RIP1 and RIP3 then form a cytosolic, pro-necrotic complex, termed complex II, which activates the pro-necrotic pathways described above [57]. Likewise, RIP1 deacetylation by sirtuin-2 (SIRT2) was also recently shown to facilitate the interaction of RIP1 with RIP3 and thus the formation of complex II [58]. This study also showed that SIRT2 deletion or inhibition also reduced I/R injury and LV dysfunction in mice and restored RIP1 acetylation, supporting the therapeutic potential for modulating RIP-mediated necrosis in ischemic heart disease.

Given the key mechanistic roles of RIP binding partners in determining cell fate, identifying novel members of the RIP interactome or necrosome has become a highly active area of research. More specifically, while RIP1 is thought to activate RIP3 via direct interaction, the substrates of activated RIP3 are essentially unknown. Recently, Wang *et al.* reported that phosphoglycerate mutase family member 5 (PGAM5), a mitochondrial protein phosphatase, is a novel interaction partner and substrate of RIP3 that serves to integrate death receptor-level signaling with mitochondrial dysfunction and mitochondria-mediated programmed necrosis [59]. The authors show that, in response to multiple necrosis inducers, PGAM5 splice variants PGAM5S and PGAM5L form a tethering complex for RIP1/RIP3 at the mitochondrial outer membrane. PGAM5 then dephosphorylates the mitochondrial fission regulator Drp1, which allows for Drp1 dimerization and thus activation. Dynamic regulation of mitochondrial fission and fusion has been implicated in both normal cardiac physiology and conditions such as ischemia and HF [60], potentially providing new options for therapeutic intervention [61,62]. Moreover, the findings of Wang *et al.* build upon previous studies, including those from our group, that have implicated mitochondria as a nexus of necroptotic signaling via RIP activity [63]. Further investigation of RIP-mediated mitochondrial fragmentation as a novel component of programmed necrosis is therefore warranted.

Perhaps the most compelling evidence for the functional role of RIP kinases in necrotic death are studies with necrostatins, a family of small-molecule RIP kinase inhibitors. Originally utilized in a cerebral ischemia model and in the molecular identification of RIP1 kinase [49,64], necrostatin has been proposed as a cardioprotective agent via targeted

inhibition of RIP1 [65]. To-date, two studies have directly examined this hypothesis in vivo. Smith *et al.* reported a significant reduction in post-I/R infarct size in mice treated with necrostatin via intraperitoneal injection at reperfusion, as well as a reduction in oxidant stress-induced mPTP opening in isolated myocytes [65]. Additional work from this group showed that cardioprotection by necrostatin required CypD, implicating mPTP modulation as a key mechanistic effector of necroptosis [66]. More recently, Oerlemans *et al.* independently demonstrated a reduction in infarct size with necrostatin delivered pre-reperfusion, as well as a decrease in chronic adverse remodeling [67]. These studies further support the potential of RIP kinase inhibition as a therapeutic strategy for ischemic injury.

Mitochondrial Calcium Uptake: A Dangerous Consequence of Ca²⁺ Overload

Cytosolic Ca²⁺ overload is a pathological hallmark of ischemic heart disease, cardiomyopathy and heart failure. Mitochondria are known to possess an extraordinary capacity to take up Ca²⁺ and may buffer cytosolic Ca²⁺ levels. However, only recently, with the molecular identification of the mitochondrial calcium uniporter (MCU) and the MCU-associated regulators mitochondrial calcium uptake 1 (MICU1) [68,69] and mitochondrial calcium uniporter regulator 1 (MCUR1) [70] has mitochondrial Ca²⁺ uptake emerged as a key mechanism of cardiovascular disease pathogenesis [23] [71]. Regulation of overall Ca²⁺ handling and Ca²⁺ compartmentalization by mitochondria are highly dynamic events, and while mitochondrial buffering of cytosolic Ca²⁺ has been proposed as an adaptive response to stress, excess mitochondrial Ca²⁺ considerably increases mPTP susceptibility and promotes myocyte death via apoptosis and necrosis [1] [72] [73]. As a result, the signaling mechanisms that regulate MCU-mediated Ca²⁺ entry into mitochondria are of considerable interest in the search for new ways to limit cardiomyocyte death.

Kinase Regulation of MCU-mediated Ca²⁺ Uptake: CaMKII at the Mitochondria

Ca²⁺/calmodulin-dependent kinase II (CaMKII) is a multifaceted serine-threonine kinase consisting of four major isoforms (α , β , γ , and δ) with several subclasses, and serves as a nodal regulator of Ca²⁺-mediated signaling and cellular Ca²⁺ cycling and homeostasis. In the heart, inhibition of CaMKII protects against I/R injury [74] and pressure overload-induced cardiac decompensation [75] and limits contractile dysfunction in failing hearts [76]. Indeed, the diversity and importance of CaMKII-mediated pathways and effectors has resulted in considerable focus on CaMKII; both as a key mediator of cardiomyocyte (patho)physiology [77] and as a potential therapeutic target [78] [79].

CaMKII has been shown to enhance cardiomyocyte death via several mechanisms [80–83]. These and other studies have revealed that CaMKII activity in the setting of I/R is strongly associated with elevation of cytosolic Ca²⁺ via increased sarcoplasmic reticulum (SR) Ca²⁺ release as well as regulation of the sodium-calcium exchanger (NCX). Often, the net result of this cytosolic Ca²⁺ overload is a secondary increase in mitochondrial Ca²⁺ and subsequent cell death. A recent study by Joiner *et al.* has now also proposed direct

regulation of MCU by CaMKII phosphorylation as a means of increasing mitochondrial Ca^{2+} uptake, thus promoting ischemic injury in the heart [84]. Using transgenic expression of a membrane and mitochondria-targeted CaMKII inhibitory protein, CaMKIIN, the authors demonstrated in mice that blocking CaMKII activity at the mitochondria decreased MCU activity (I_{MCU}) and reduced Ca^{2+} -induced mPT. Moreover, targeted CaMKII inhibition reduced infarct size, mitochondrial damage and cell death in a mouse model of I/R. These data suggest that therapeutic CaMKII inhibition could serve to limit mitochondrial Ca^{2+} overload and subsequent cell death. These findings reveal a previously unexplored role for CaMKII signaling at the mitochondria, as well as raise important additional questions [85]. In particular, the mechanisms by which CaMKII translocates to the mitochondria, and the ways in which mitochondrial CaMKII is activated (it is still unclear whether mitochondria contain a functional pool of calmodulin) have not yet been determined. Activation of CaMKII by oxidative modification is one possible mechanism [86]. Indeed, CaMKII oxidation has been shown to regulate cardiac dysfunction in response to stress [87,88], raising the possibility that CaMKII oxidation may also occur at the mitochondria, a central location of redox signaling.

Despite the considerable gains made toward understanding mitochondrial Ca^{2+} import, the full molecular composition of the MCU remains undefined. Using an RNAi-based screen, the Madesh lab recently identified mitochondrial calcium uniporter regulator 1 (MCUR1), as a novel and essential component of MCU-mediated Ca^{2+} uptake [70]. More recently, Plovanich *et al.* reported on the existence of MICU1 and MICU2, two paralogous forms of the MCU regulator MICU1 [89]. These studies demonstrated a dynamic regulation of MCU function by these component proteins, raising the possibility that kinase signaling via CaMKII (or other kinases) could also indirectly impact MCU activity via regulation of MCU-interacting proteins. The study by Joiner *et al.* presents a novel regulatory role for CaMKII in subcellular Ca^{2+} handling; thus further studies are warranted to further explore the functional consequences of mitochondrial CaMKII signaling and potential therapeutic applications. Of note, as in the case of GSK-3, targeting CaMKII for inhibition will also likely necessitate the development of highly isoform-specific inhibitors, as the multiple CaMKII subtypes can often play divergent, if not contrasting, roles, both in the heart and elsewhere [90,91].

Conclusions: Targeted Protein Kinase Inhibition for Ischemic Heart Disease

Herein we have discussed three emerging mechanisms of cell death in the ischemic heart: mPTP opening, programmed necrosis, and Ca^{2+} overload, as well as provided an example of a nodal kinase pathway for each, inhibition of which may represent a therapeutic strategy for limiting myocyte loss. We have not attempted to cover the myriad other kinases implicated in the regulation of these cell death mechanisms. Rather, we have chosen to highlight recent developments, as well as emphasize the convergence of these pathways on the mitochondria, which has become an increasingly crucial contributor to multiple cell death programs.

Kinase inhibitors have remained an attractive therapeutic strategy for many diseases; however, their clinical application for cardiovascular disease, despite much enthusiasm, has languished, undoubtedly due in part to the hurdles involved in inhibitor development [92] as

well as the complex nature of heart disease. This underscores the continued importance of extensively validating potential targets, particularly newer targets such as RIP1/RIP3, in well-defined animal models [93]. Moreover, the need to develop and validate isoform-specific inhibitors for targets such as GSK-3 and CaMKII remains a crucial step in the transition to the clinic. As such, the kinases reviewed here are promising examples of drug targets warranting further studies, to determine their suitability and applicability for clinical application.

Acknowledgments

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Figure 1. mPTP-mediated Cell Death and Regulation by Akt-GSK3 Signaling

Kinases implicated in mitochondrial permeability transition pore (mPTP) opening downstream of Akt activation. Cyclophilin D (CypD) is an essential regulator of mPTP. Other proposed molecular contributors to mPTP include the voltage-dependent anion channel (VDAC), the adenine nucleotide transporter (ANT), and the mitochondrial phosphate carrier (PiC).

Figure 2. Programmed Necrosis and Regulation by The RIP Kinase Pathway

Schematic depicting the selective assembly of the necrosome/necroptosome under I/R or inflammatory (Infl.) stress. Apoptotic versus necrotic outcome is governed by the maintenance of RIP1 and RIP3 kinase levels and activity, as well as the subcellular recruitment of the RIP1/RIP3 complex to the membrane versus the cytosol and mitochondria.

Figure 3. Mitochondrial Calcium Overload and Regulation by CaMKII

Sources of cytosolic Ca^{2+} overload in response to I/R, and modulation by Ca^{2+} /calmodulin-dependent protein kinase (CaMKII). CaMKII has recently also been proposed to impact mitochondrial Ca^{2+} uptake via the mitochondrial calcium uniporter (MCU), which is itself regulated by mitochondrial calcium uptake 1 (MICU1) and mitochondrial calcium uniporter regulator 1 (MCUR1).