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The Latest Waves in Calcium Signaling

Meeting Report

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Ca^{2+} is a universal second messenger that is a key component of myriad processes in all cell types. Perturbations in normal intracellular Ca^{2+} concentrations underlie many common pathological conditions, ranging from cardiac hypertrophy to ischemic death of neurons. A recent meeting addressed the contributions of Ca^{2+} and Ca^{2+} binding proteins to health and disease. Insights gleaned from mechanistic studies offered the potential for new therapeutic approaches to combat a variety of diseases resulting from alterations in Ca^{2+} homeostasis.

The 14th International Symposium on Ca^{2+} and Ca^{2+} Binding Proteins in Health and Disease (Banff, Canada, April 5–10, 2005) opened with a tribute by Anthony Means (Duke University) in memory of Yasutomi Nishizuka. Nishizuka is best known for his landmark discovery of protein kinase C (PKC), which requires both Ca^{2+} and diacylglcerol for its activity (Takai et al., 1977, 1979). This discovery was remarkable because it demonstrated that a common lipid, diacylglcerol, is a key component of cell signaling and that synergy exists between Ca^{2+} and lipid second messengers. Of equal significance was the realization by Nishizuka and colleagues that PKC is a target of tumor-promoting phorbol esters (Castagna et al., 1982). Thus, PKC participates in both normal cellular signaling and in pathological conditions, including tumor formation.

The notion that the same signaling molecule (in this case, Ca^{2+}) can be the nexus for normal cell signaling as well as a contributor to aberrant physiology and disease arose many times throughout the meeting. As enunciated by keynote speaker Ernesto Carafoli (University of Padova), "Ca^{2+} is ambivalent" in the sense that changes in Ca^{2+} concentration can lead to any of a plethora of physiological consequences, including cell death (Carafoli, 2005).

There are several notable reasons why Ca^{2+} is universally used throughout phylogeny and by all cell types for signal transduction. There is ~10,000-fold difference in Ca^{2+} concentration across the plasma membrane (1.5 mM outside the cell and 0.1 μM inside), which drives Ca^{2+} into the cell through Ca^{2+}-permeable influx channels. There are also considerable but more variable Ca^{2+} gradients across the membranes of certain organelles, such as the endoplasmic reticulum (ER) and mitochondria. Also key for a second messenger, Ca^{2+} is small and diffuses rapidly.

Ca^{2+} also displays a variety of additional characteristics, as pointed out by Carafoli and the second keynote speaker, Michael Berridge (The Babraham Institute), that enable this ion to serve as the most versatile of all signaling molecules (Berridge et al., 2003). These include the ability of Ca^{2+} to autoregulate its own concentration and physiological effects, and to operate over a wide dynamic range and a variety of time scales. Spatially and temporally controlled changes in Ca^{2+} concentration are central to the ability of this messenger molecule to regulate processes ranging from synaptic transmission to apoptosis, muscle contraction, fertilization, cell division, and sensory signaling. Ca^{2+} regulates these cellular events by modulating protein degradation, transcription, cytoskeletal organization, protein phosphorylation, exo- and endocytosis, and many other processes.

Given the traditional emphasis of this meeting on the molecular mechanisms underlying Ca^{2+} signaling, it was notable that one presentation after another exemplified successes in translating basic mechanistic concepts into a deeper understanding of human health and disease. A few vignettes illustrating this theme, with a particular emphasis on cardiac and neuronal diseases, will be described. First, it is worth recounting some of the important developments concerning the nature of what Berridge refers to as the "Ca^{2+} signaling toolkit" (Berridge et al., 2003) and how these molecules contribute to spatially and temporally controlled signaling (see Figure 1).

Ca^{2+} Influx Channels

The sources of Ca^{2+} are the extracellular milieu and certain intracellular organelles, such as the ER and the mitochondria. One class of influx channels, the voltage-operated Ca^{2+} channels (VOCCs), includes $Ca_V2.1$ (P/Q type α-1A). Terry Snutch (University of British Columbia) pointed out how this channel, but not other VOCCs, leads to activation of the SNARE protein syntaxin-1A. It turns out that the carboxyl terminus of $Ca_V2.1$ is the critical region required for this activity, as fusion of this region to other VOCCs leads to transcription of the syntaxin gene. The scaffold protein Homer also contributes to this transcriptional activation, as deletion of Homer binding sites prevented the induction of syntaxin transcription. Though the requirement for the interaction of $Ca_V2.1$ with Homer remains unclear, the results suggest that localized entry of Ca^{2+} through this channel, perhaps mediated by interactions with Homer, may be necessary for transcription of the syntaxin gene.

The VOCCs have ancient origins, as Kyle Cunningham (Johns Hopkins University) pointed out. For example, a related VOCC channel in yeast is activated by high pH shock and mating pheromones, and inactivation of the yeast VOCC occurs in part by dephosphorylation of the catalytic subunit (Cch1) by calcineurin. To identify additional proteins that regulate the yeast VOCC, Cunningham conducted a genetic screen for mutations that resulted in the activation of the VOCC in

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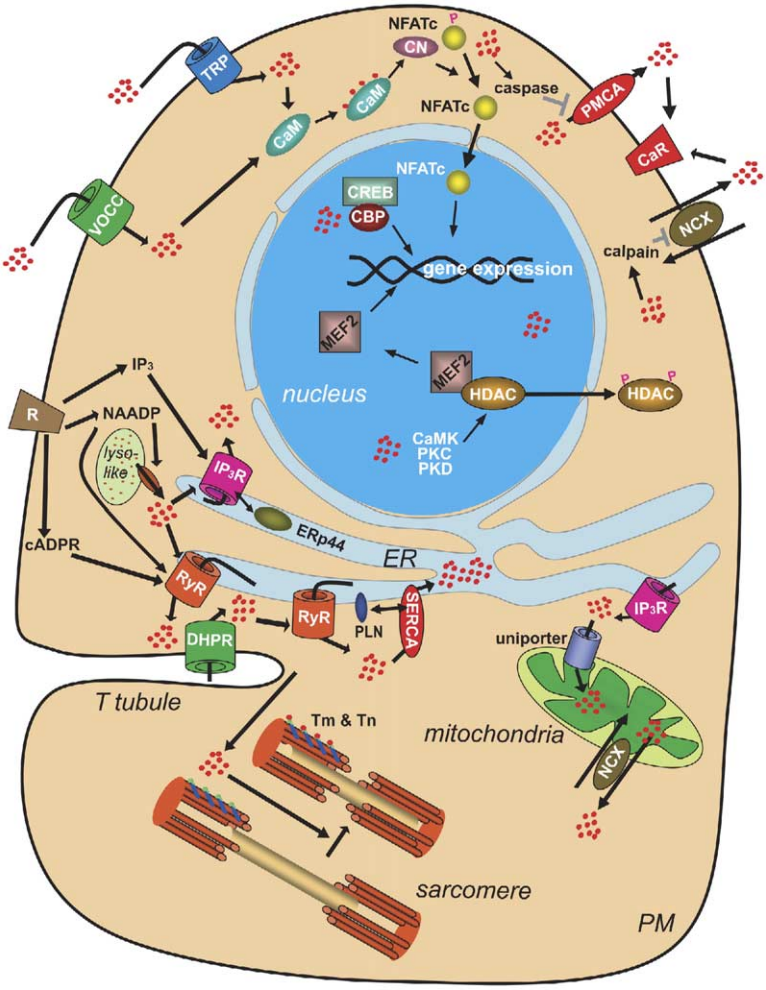

Figure 1. Components of the Ca^{2+} Signaling Toolkit

A hybrid cell is depicted showing the Ca^{2+} signaling proteins present in muscle cells, neurons, and nonexcitable cells. This figure illustrates the multiple modes through which Ca^{2+} can enter and leave the cell and the variety of Ca^{2+} storage organelles that take up and release Ca^{2+}. There is significant crosstalk between these various organelles. Also indicated are some of the effects of Ca^{2+} on gene expression and contraction of the sarcomere. Ca^{2+} ions are represented by small red dots. cADPR, cyclic ADP-ribose; CaM, calmodulin; CaMK, calmodulin-dependent protein kinase; CaR, extracellular calcium-sensing receptor; CN, calcineurin; CREB, cAMP response element binding protein; DHPR, dihydropyridine receptor; HDAC, histone deacetylase; IP_3, inositol triphosphate; IP_3R, IP_3 receptor; MEF2, myocyte transcription factor 2; NAADP, nicotinic acid adenine dinucleotide phosphate; NCX, Na^+/Ca^{2+} exchanger; NFAT, nuclear factor of activated T cells; PKC, protein kinase C; PKD, protein kinase D; PLN, phospholamban; PM, plasma membrane; PMCA, plasma membrane Ca^{2+} ATPase; R, either a G protein-coupled receptor or a receptor tyrosine kinase that leads to production of either IP_3, cADPR, or NAADP; RyR, ryanodine receptor; SERCA, sarcoplasmic and endoplasmic reticulum calcium ATPase; Tm, tropomyosin; Tn, troponin.

the absence of other activating stimuli. Among the genes isolated were those encoding the proteins Mid1 and Ecm7, which are related to the $\alpha_2\delta$ and γ regulatory subunits of vertebrate VOCCs. The third protein, Cch1, binds to and stabilizes Mid1. Interestingly, another γ subunit seems to stimulate a new Ca^{2+} influx channel.

Another class of Ca^{2+} influx channel, the TRP channels, was discussed in several talks. David Clapham (Harvard Medical School) explained that TRPC5-dependent cation influx is activated in the neurites of nerve cells through a mechanism that involves regulated translocation of the channel to the plasma membrane. This shuttling requires the activity of a PI-3-kinase and Rac1 and leads to inhibition of neurite outgrowth. This work is important, as it indicates, along with related work in the field, that TRP-dependent influx can be regulated through dynamic movement to the plasma membrane (Montell, 2004), in addition to a more classical mechanism involving opening of the pore in channels already situated in the cell cortex.

Regulated movement of TRP channels was further emphasized by Don Gill (University of Maryland School of Medicine). He described his collaborative study with Sol Snyder (Johns Hopkins University School of Medicine) demonstrating that surface expression and influx activity of a related channel, TRPC3, is mediated by a lipid binding pleckstrin homology (PH) domain. The exciting and unexpected aspect of this work is that only half of the PH domain is present on TRPC3, while the other half is present on the interacting protein, PLCγ. Thus, lipid-mediated surface expression is dependent on the interaction of these two molecules. These data raise the intriguing possibility that the presence of PH domains, split between two proteins, may be a more common mechanism regulating the membrane localization of proteins than previously appreciated.

Ca^{2+} Release and Reuptake

Release of Ca^{2+} from intracellular stores, a focus of many presentations at the meeting, is the other major event that affects Ca^{2+} concentration in the cytoplasm. Notably, release of Ca^{2+} from internal stores can lead to an increase in the extrusion of Ca^{2+} from the cell. As a result, there is a small localized increase in extracellular Ca^{2+}, which may be physiologically relevant. Ca^{2+} has the highly unusual but not so widely appreciated ability to act as an extracellular messenger in addition to its well recognized role as an intracellular second messenger (Hofer and Brown, 2003). The extracellular Ca^{2+} sensor is a G protein-coupled receptor (CaR) in the plasma membrane. Inactivating and activating mutations in the CaR underlie several hyper- and hypocal-

cemic disorders, respectively (Bai, 2004), although the signaling pathways coupled to CaR are poorly understood. Aldebaran Hofer (Harvard Medical School) summarized her work with Silvana Curci (Harvard Medical School) showing that the CaR modulates the secretory activity of gastric cells in the intact gut mucosa. Moreover, their studies suggest that the signaling cascade downstream of CaR depends on coupling of this channel to G_i and reduction in cAMP levels.

The most extensively studied intracellular Ca^{2+} stores are those that release Ca^{2+} through either the ryanodine receptor (best characterized in cardiac and skeletal muscle cells) or the widely expressed IP_3 receptor (IP_3R). Katsuhiko Mikoshiba (University of Tokyo) described his work demonstrating that dynamic movements of the ER, and consequently of the IP_3Rs, are regulated in part through interactions with the microtubule-based motor kinesin. Among the many fascinating stories recounted by Mikoshiba is his recent identification of the ERp44 protein present in the ER lumen. This protein contains a thioredoxin homology domain (THD) and binds to and negatively regulates the activity of the IP_3R channel. Interestingly, the interaction of ERp44 with IP_3R is favored by a relatively low Ca^{2+} concentration in the ER as well as by reducing conditions, indicating that dynamic changes in the ER milieu regulate the activity of IP_3R.

In addition to IP_3, two other Ca^{2+}-mobilizing messengers, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), were described in presentations by Antony Galione (Oxford University), Ole Petersen (University of Liverpool), and Oleg Gerasimenko (University of Liverpool). Although these two agents have received less attention than IP_3, NAADP is far more potent in stimulating Ca^{2+} release. Ca^{2+} mobilization induced by cADPR and NAADP is important for processes ranging from sperm activation to insulin secretion, from muscle contraction to fluid secretion (Galione and Petersen, 2005; Guse, 2004). The coordinated actions of NAADP, cADPR, and IP_3 contribute to repetitive increases and decreases in intracellular Ca^{2+} concentration through Ca^{2+}-induced Ca^{2+} release. The trigger for this Ca^{2+}-induced Ca^{2+} release could be Ca^{2+} release mediated by NAADP, which in turn mobilizes Ca^{2+} via IP_3- and cADPR-sensitive channels. Interestingly, various acidic lysosomal-like organelles may represent one type of NAADP-sensitive Ca^{2+} pool. Both the Galione and Petersen laboratories report that these organelles contain ryanodine receptors or are in close apposition to Ca^{2+} stores containing ryanodine receptors. A current challenge for the field is to identify the Ca^{2+} release channel that is sensitive to NAADP. Galione's work indicates that the relevant channel is distinct from the IP_3R and the ryanodine receptors, whereas data from Petersen and Gerasimenko show that NAADP activates ryanodine receptors.

Although mitochondria are a major Ca^{2+} store, the contribution of these organelles to Ca^{2+} signaling was largely ignored until the work of Rosario Rizzuto (University of Padova) and Tullio Pozzan (University of Padova), who both spoke at the meeting. They showed that Ca^{2+} release from IP_3-sensitive Ca^{2+} stores in the ER prompted a rise in the luminal Ca^{2+} concentration of neighboring mitochondria (Rizzuto et al., 1993). This work, illustrating the close association of ER and mitochondrial Ca^{2+} stores, has been pivotal in renewing interest in mitochondria among Ca^{2+} signaling researchers.

Mitochondria are not static structures but comprise a large tubular network, which undergoes dynamic structural changes through fission and fusion. However, the physiological roles of mitochondrial reorganization remain unclear. To address this question, Rizzuto, Nicolas Demaurex (University of Geneva), and their colleagues took advantage of the ability to fragment mitochondria by overexpressing either the dynamin-related protein (Drp-1) or a mitochondrial outer membrane protein (hFis1) that recruits and thereby promotes fragmentation by Drp-1. Fragmentation of mitochondria, and the subsequent retraction of cortically located mitochondria from the plasma membrane, had no effect on the transfer of Ca^{2+} from the ER to mitochondria. However, fragmentation did reduce the rapid and coordinated propagation of the Ca^{2+} rise within the mitochondria resulting in an increase in Ca^{2+} concentration that was discrete and sequential. Given the importance of the spatial and temporal control of Ca^{2+} signaling in cells, the state of the mitochondrial network is likely to have important physiological consequences. In fact, as Rizzuto noted, fragmentation of mitochondria results in profound suppression of ceramide-induced cell death.

The yin of Ca^{2+} release is, of course, counterbalanced by the yang of Ca^{2+} reuptake. The best-characterized protein involved in Ca^{2+} reuptake is the Ca^{2+}-ATPase referred to as SERCA (sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase). Ca^{2+}-ATPases are pumps that couple the energy released from hydrolysis of ATP to the transport of Ca^{2+} against a concentration gradient. During excitation-contraction coupling in muscle, release of Ca^{2+} from the sarcoplasmic reticulum (SR) via ryanodine receptors results in muscle contraction, whereas reuptake of Ca^{2+} via the SERCA causes the opposite effect, muscle relaxation. Elucidating the mechanisms of action of the ryanodine receptor and the Ca^{2+}-ATPase are critical for understanding not only normal heart physiology but also for deciphering the basis for a variety of cardiac pathologies. There are multiple defined states of the SERCA that differ in terms of affinity and accessibility of Ca^{2+} and interactions with adenosine phosphates and Mg^{2+}. Chikashi Toyoshima (University of Tokyo) described his work, a real tour de force, concerning the high-resolution structures of five states of the SERCA pump. The results are important, as they reveal large structural rearrangements within the transmembrane and cytoplasmic domains of the SERCA that facilitate the pumping of Ca^{2+}. Among many insights is the conclusion that ADP release is crucial for opening the gate leading to entry of Ca^{2+} into the sarcoplasmic reticulum lumen.

Making Waves In Vivo

The coordinated release and uptake of Ca^{2+} from intracellular stores that occurs during Ca^{2+}-induced Ca^{2+} release generates oscillatory changes in the Ca^{2+} concentration that sweep across the cell. Such waves of Ca^{2+} regulate a daunting array of physiological consequences, starting with the earliest cellular event, fertil-

ization. As described by Mikoshiba, it is now well established that entry of sperm into an egg induces the propagation of Ca^{2+} waves within the egg through release from IP_3-sensitive Ca^{2+} stores and that this process is critical for fertilization. At the meeting, Luigia Santella (Stazione Zoologica Anton Dohrn) described new insights into the early events in the production of these Ca^{2+} waves using starfish oocytes. It appears that the initial rise in Ca^{2+} concentration is due to influx of the cation via plasma membrane channels that are activated by NAADP. Cortical Ca^{2+} release quickly follows, thereby initiating the Ca^{2+} waves. Thus, at least in the starfish, NAADP-induced Ca^{2+} influx is the initiating event in fertilization. The nature of the influx channel remains to be identified, though Santella proposed TRP channels as potential candidates.

Ca^{2+} waves are not restricted to single cells but can be coordinated among many neighboring cells via gap junctions. However, it has been a challenge to monitor these coordinated Ca^{2+} waves in vivo and to ascertain their functions. Arthur Konnerth (University of Munich) presented his data with Ca^{2+} imaging techniques, which he used to monitor Ca^{2+} waves in the brains of live mice. Ca^{2+} waves were detected in the developing cortex of postnatal animals as well as in adults, where slow waves were evoked by sensory input, such as auditory stimulation or light. The measurement of these waves—referred to as sensory-evoked compound Ca^{2+} transients (cCaTs)—offers the possibility of building cortical maps based on these activities. Moreover, the cCaTs may represent a previously unknown response characteristic of each sensory modality.

Characterizing Macromolecular Assemblies

Given that Ca^{2+} modulates a diverse array of cellular processes, a key question concerns the molecular bases for specificity in signaling. In some cases, specificity seems to be generated by a localized rise in intracellular Ca^{2+} concentration, whereas in other instances it is achieved through the formation of signaling complexes. Shmuel Muallem (The University of Texas Southwestern Medical Center) described his latest work in characterizing a molecular scaffold protein referred to as spinophilin. This scaffold is coupled to and regulates Ca^{2+} signaling by associating with G protein-coupled receptors, such as the α-adrenergic receptor (αAR), and with RGS (regulator of G protein signaling) proteins. RGS proteins promote the GTPase activity of the Gα subunit and thereby attenuate GPCR signaling. Muallem found that reduction of αAR-mediated Ca^{2+} signaling occurs by direct binding of spinophilin to RGS and recruitment of RGS to the scaffold complex.

A key question relevant to macromolecular assemblies concerns the relative spatial organization of the components in vivo. Trisha Davis (University of Washington) addressed this issue in yeast, focusing on an important calmodulin-containing complex. One of the many functions of calmodulin is in the microtubule-organizing center called the spindle pole body, which is the yeast equivalent of the centrosome in metazoan organisms. Calmodulin is present in the core of the spindle pole body, along with four other proteins, though the spatial arrangements of these proteins in vivo have been difficult to define. Davis described a creative variation of FRET analysis that more accurately assayed energy transfer than previous approaches. She used available cryo-EM data in combination with the new FRET metric, which relied heavily on carefully designed controls to reduce problems with spillover from the cyan fluorescent protein and yellow fluorescent protein channels. Davis and her colleagues were able to construct a detailed lattice structure of the spindle pole body consisting of calmodulin and the other components of the central plaque, which is one of the two core layers of the spindle pole body. This work is exciting because this new FRET metric can be applied to further define the arrangements of other large protein complexes in vivo.

Calcineurin and the Regulation of Transcription

Fluctuations in the Ca^{2+} concentration in the nucleus can have profound effects on gene expression both in excitable cells such as neurons and in nonexcitable cells. As discussed at the meeting by Hilmar Bading (University of Heidelberg), in neurons, the nuclear Ca^{2+} concentration is a key regulator of both neuronal plasticity and cell survival. One of the mechanisms by which nuclear Ca^{2+} affects gene expression is through Ca^{2+}/calmodulin regulation of enzymes that control the phosphorylation states of proteins. These enzymes include protein kinases such as calmodulin-dependent protein kinases II and IV (CaM kinase II and IV) as well as the protein phosphatase calcineurin, which is responsible for dephosphorylating proteins. Anthony Means (Duke University) described his work on CaM kinase IV knockout mice, which exhibit cerebellar defects due to changes in cerebellar granule cell proliferation and migration, as well as alterations in the hematopoietic progenitor cell population resulting in stem cell exhaustion upon transplantation to recipient mice.

As outlined in several talks in a calcineurin workshop chaired by Claude Klee (National Cancer Institute), calcineurin dephosphorylates the NFAT proteins c1–c4, inducing their translocation to the nucleus and their assembly into NFAT complexes that activate or repress transcription. Gerald Crabtree (Stanford University) described his studies of mutant mice that revealed the importance of calcineurin/NFAT signaling in development. The developmental roles of the genes in this pathway include cardiovascular, lung, skeletal and kidney morphogenesis, and axonal outgrowth as well as lineage specification within the hematopoietic and nervous systems. Anjana Rao (Harvard Medical School) described a genomewide RNAi screen in *Drosophila* to identify regulators of the Ca^{2+}/calcineurin/ NFAT signaling pathway. One of the candidates to emerge from this screen is STIM1, an EF hand protein that may serve as the ER sensor for store-operated Ca^{2+} channels, as its knockdown abolishes thapsigargin- or receptor-mediated Ca^{2+} influx (Liou et al., 2005; Roos et al., 2005).

Though NFAT is expressed only in vertebrate organisms, a role for calcineurin in mediating Ca^{2+}-dependent transcription is conserved from yeast to humans. Patrick Hogan (Harvard Medical School) described a conserved docking site on calcineurin used by several of its substrates including NFAT and the yeast tran-

scription factor Crz1p. Martha Cyert (Stanford University) recounted her work demonstrating that, in the yeast *Saccharomyces cerevisiae*, calcineurin and Crz1p are essential for responses to environmental stress, such as elevations in pH, temperature, or salinity. Cyert and colleagues have identified three additional substrates that bind to and are dephosphorylated by calcineurin. These include two proteins, Slm1p and Slm2p, that associate with the plasma membrane via PH domains and regulate the actin cytoskeleton. The third substrate, Hph1p, is present in the ER and is a component of the secretory pathway. These data illustrate roles for calcineurin in responding to environmental stress through both transcriptional and post-transcriptional mechanisms.

Ca^{2+} and Pathological Neuronal Death

It is well established that excessive intracellular Ca^{2+} can contribute to neuronal cell death. However, Ca^{2+} overload is generally avoided through the activities of ATP-driven pumps (plasma membrane Ca^{2+} ATPase; PMCA) and Na^+/Ca^{2+} exchangers (NCX and NCKX). The widely expressed NCX couples the removal of one Ca^{2+} ion with entry of three Na^+ ions; the eye- and brain-enriched NCKX extrudes one Ca^{2+} and one K^+ ion in exchange for four Na^+ ions. In general, PMCAs have relatively high affinities for Ca^{2+}, imbuing them with the ability to respond to relatively small increases in Ca^{2+} concentration. The lower affinity exchangers have higher transport rates, enabling them to more quickly expel Ca^{2+} from cells. Carafoli recounted his collaborative work with Pierluigi Nicotera (University of Leicester) concerning the caspase-dependent cleavage of the PMCA near its carboxyl terminus, and the calpain-induced cleavage of the NCX, which can lead to Ca^{2+} overload. The PMCA is cleaved in neurons in response to excitotoxins leading to apoptosis; expression of a noncleavable PMCA derivative protects against death of neurons. The NCX is also cleaved in response to excitotoxicity, promoting cell death. Analogous to the PMCA story, inhibition of NCX cleavage, either by expression of a calpain inhibitor or a noncleavable form of NCX, prevented Ca^{2+} overload and excitotoxic cell death. These findings have potential therapeutic significance, as inhibition of the PMCA or NCX cleavage may reduce neuronal cell death resulting from the Ca^{2+} overload associated with brain ischemia and other excitotoxic conditions.

Hypoxic conditions, such as those that occur during ischemia, lead to activation of at least two types of Ca^{2+}-permeable channels in neurons, one of which is the NMDA receptor. Bading discussed how activation of NMDA receptors in synaptic versus extrasynaptic locations can lead to pro- or antisurvival consequences, respectively. The extrasynaptic activation of NMDA receptors antagonizes signaling to the Ca^{2+}-activated transcription factor CREB, which is normally activated by synaptic NMDA receptors. Bading is profiling the genes that are up- or downregulated by signaling through NMDA receptors situated in or outside of synaptic regions and has found that many of the genes are distinct. These results demonstrate that the effects of Ca^{2+} signaling on neuronal gene expression and survival can be quite different, depending on the subcellular localization of the activated channels.

In addition to the NMDA receptor, several types of mammalian and *Drosophila* TRP channels are activated by hypoxic conditions. It is possible that much of the cell death that arises from ischemia during stroke results from extended activation of TRP channels. Craig Montell (Johns Hopkins University School of Medicine) showed that the neuronal cell death due to constitutive activation of fruit fly TRP could be largely suppressed by increasing the activity of the Na^+/Ca^{2+} exchanger. These results underscore the possibility that manipulation of Na^+/Ca^{2+} exchanger activity can have profound effects on ameliorating neuronal cell death resulting from Ca^{2+} overload.

Getting at the Heart of Cardiac Disease

One of the major advances in the Ca^{2+} signaling field has been the flurry of new insights into cardiac function and the molecular bases of cardiac hypertrophy, arrhythmia, and heart failure. More than half of patients with heart failure die suddenly, and ventricular arrhythmia is the leading cause. This sudden cardiac death is typically induced by stress, either physical or emotional. Unfortunately, current treatments are of limited value.

Among the many fascinating presentations at the meeting was one by Wayne Chen (University of Calgary), who discussed a trigger for cardiac arrhythmia, which he termed store-overload-induced Ca^{2+} release (SOICR). Normally, muscle cell depolarization induces release of Ca^{2+} from the sarcoplasmic reticulum and subsequent muscle contraction. It has been established by others that SOICR occurs when Ca^{2+} overload in the sarcoplasmic reticulum induces muscle contraction in the absence of depolarization, leading to cardiac arrhythmia. Chen and coworkers now provide strong evidence that mutations in the cardiac ryanodine receptor, which are linked to ventricular tachycardia and sudden death, reduce the threshold for SOICR. Furthermore, these mutations appear to reduce the activation threshold by increasing the channel sensitivity to luminal Ca^{2+}. These results are very exciting, as they raise the intriguing possibility that therapies that reverse the sensitivity of ryanodine receptors to SOICR could provide a new strategy for treating ventricular arrhythmias.

Defects in heart function also occur as a result of mutations that alter the activity of SERCA, as reuptake of Ca^{2+} into the sarcoplasmic reticulum leads to relaxation of cardiac muscle. A key modulator of SERCA is a small single transmembrane protein, phospholamban (PLN), which binds to and lowers the affinity of SERCA for Ca^{2+}, thereby inhibiting its ability to promote reuptake of Ca^{2+} into the sarcoplasmic reticulum. David MacLennan (University of Toronto) described the structural and biochemical studies from his and Toyoshima's laboratories, which have illuminated the basis by which PLN inhibits SERCA. In addition, MacLennan and colleagues have shown that a protein related to PLN, referred to as sarcolipin (SLN), is expressed in skeletal and atrial muscles, where it carries out a similar regulatory function. These and other studies recounted by

MacLennan form the basis for understanding how mutations in PLN that increase or decrease its inhibitory activity contribute to cardiomyopathy. For example, SERCA Ca^{2+} affinity and activity is increased in hearts from PLN knockout mice and decreased in transgenic animals expressing superinhibitory forms of PLN. The muscles of PLN-deficient mice also show greater contractility, whereas the converse is observed as a result of overexpression of superinhibitory PLN. Notably, MacLennan recounted his collaborative studies with Jon and Christine Seidman (Harvard Medical School) and Litsa Kranias (University of Cincinnati) associating mutations in PLN with cardiac failure in humans. Furthermore, cardiac overexpression of SLN in transgenic mice formed a superinhibitory PLN-SLN complex that reduced SERCA function and muscle contractility. These studies on PLN and SLN are exciting, as they raise the possibility that therapies that modulate SERCA activity might reduce the probability of heart failure in individuals with either increased or decreased SERCA function.

Contraction of skeletal and cardiac muscle is regulated by Ca^{2+} through interactions with actin-associated proteins, including tropomyosin and troponin C. This latter protein is a member of the troponin (Tn) complex and consists of four Ca^{2+} binding EF hands. There are two other members of the Tn complex, TnI (inhibitory subunit) and TnT (tropomyosin binding subunit), and mutations in each of the three cardiac troponin proteins have been shown to underlie familial hypertrophic cardiomyopathy. Brian Sykes (University of Alberta) presented his work in which he has used NMR measurements to compare the structural consequences resulting from several mutations in TnI that underlie certain forms of familial hypertrophic cardiomyopathy. In normal muscle cells, high Ca^{2+} relieves the inhibition caused by the tropomyosin-troponin complex, allowing the myosin to slide along the actin and promote contraction. In addition, phosphorylation of TnI mediated by protein kinase A affects its interaction with TnC. Sykes and colleagues have shown that several of the familial hypertrophic cardiomyopathy mutations in TnI alter the effects of phosphorylation on the interaction of TnI with TnC.

A potentially new approach for ameliorating pathological cardiac hypertrophy was outlined in a presentation by Eric Olson (The University of Texas Southwestern Medical Center). Cardiac stress leads to reactivation of fetal genes, which in turn causes an increase in the size of cardiomyocytes, reduced contractility of these muscle cells, and heart failure. The reprogramming of gene expression occurs in part through a pathway initiated by stress-induced activation of receptors that promotes a rise in intracellular Ca^{2+}. The increase in Ca^{2+} activates protein kinases that phosphorylate two conserved serines in histone deacetylases (HDACs), which otherwise bind to and repress activity of the myocyte transcription factor 2 (MEF2). Phosphorylation of the HDACs creates docking sites for a chaperone protein and promotes translocation of the HDACs from the nucleus to the cytoplasm. As a consequence, MEF2 is free to bind to a coactivator, which has intrinsic histone acetylase activity, leading to chromatin relaxation. The cascade culminates with increased MEF2-mediated transcription, resulting in hypertrophic growth. Olson described his work demonstrating that knockout of either HDAC5 or HDAC9 sensitizes mice to stress-induced cardiac hypertrophy. Conversely, mutations in the conserved HDAC phosphorylation sites increase the resistance of mice to stress. In the case of HDAC5, phosphorylation is mediated through a pathway involving protein kinase C and protein kinase D. An exciting area of exploration by Olson and colleagues is to develop therapies that control pathological cardiac hypertrophy by promoting the repressive activity of HDACs. Such strategies include developing inhibitors of HDAC phosphorylation or drugs that stabilize the HDAC/MEF2 interaction.

The 14th International Symposium on Ca^{2+} and Ca^{2+} Binding Proteins in Health and Disease was true to its name in that there was a clear integration of basic molecular insights into Ca^{2+} signaling with issues of relevance to human health. The challenge over the next few years will be to test whether the creative ideas for combating human disease that are emerging from mechanistic insights will lead to new therapeutic approaches for treating cardiac and neurological diseases as well as other pathologies.

Acknowledgments

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References

Bai, M. (2004). Structure-function relationship of the extracellular calcium-sensing receptor. Cell Calcium *35*, 197–207.

Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. *4*, 517–529.

Carafoli, E. (2005). The Symposia on Calcium Binding Proteins and Calcium Function in Health and Disease: an historical account, and an appraisal of their role in spreading the calcium message. Cell Calcium *37*, 279–281.

Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y. (1982). Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. *257*, 7847–7851.

Galione, A., and Petersen, O.H. (2005). The NAADP receptor: new receptors or new regulation?. Mol. Interv. *5*, 73–79.

Guse, A.H. (2004). Regulation of calcium signaling by the second messenger cyclic adenosine diphosphoribose (cADPR). Curr. Mol. Med. *4*, 239–248.

Hofer, A.M., and Brown, E.M. (2003). Extracellular calcium sensing and signalling. Nat. Rev. Mol. Cell Biol. *4*, 530–538.

Liou, J., Kim, M.L., Do Heo, W., Jones, J.T., Myers, J.W., Ferrell, J.E., Jr., and Meyer, T. (2005). STIM Is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. Curr. Biol. *15*, 1235–1241.

Montell, C. (2004). Exciting trips for TRPs. Nat. Cell Biol. *6*, 690–692.

Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993). Microdo-

mains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. Science *262*, 744–747.

Roos, J., Digregorio, P.J., Yeromin, A.V., Ohlsen, K., Lioudyno, M., Zhang, S., Safrina, O., Kozak, J.A., Wagner, S.L., and Cahalan, M.D. (2005). STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J. Cell Biol. *169*, 435–445.

Takai, Y., Kishimoto, A., Inoue, M., and Nishizuka, Y. (1977). Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. I. Purification and characterization of an active enzyme from bovine cerebellum. J. Biol. Chem. *252*, 7603–7609.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., and Nishizuka, Y. (1979). Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J. Biol. Chem. *254*, 3692–3695.