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Forum Review

Zinc Dynamics in the Myocardial Redox Signaling Network

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ABSTRACT

Zinc plays a vital role in various cellular functions. Zinc deprivation is associated with severe disorders related to growth, maturation, and stress responses. In the heart, zinc affects differentiation and regeneration of cardiac muscle, cardiac conductance, acute stress responses, and recovery of heart transplants. Recent discoveries of the molecular players in zinc homeostasis revealed that the amount of intracellular free zinc is tightly controlled on the level of uptake, intracellular sequestration, redistribution, storage, and elimination, consequently creating a narrow window of optimal zinc concentration in the cells. Most of intracellular zinc is bound to numerous structural and regulatory proteins, with metabolically active, labile zinc present in pico- to nanomolar concentrations. The central position of zinc in the redox signaling network is built on its unique chemical nature. The redox inert zinc creates a redox active environment when it binds to a sulfur ligand. The reversible oxidation of the sulfur ligand is coupled to the reversible zinc release from the protein, thereby executing the task of so-called protein "redox zinc switch." Clearly, the impairment of zinc homeostasis will have far reaching physiological consequences. *Antioxid. Redox Signal.* 8, 1707–1721.

INTRODUCTION

ZINC IS ONE OF THE MOST ABUNDANT TRANSITION metals in the human body, second only to iron, and it is responsible for a wide range of biological functions. Approximately 10% of the United States population ingests <50% of the recommended daily allowance for zinc. Nutritional studies of trace elements demonstrated the necessity of the proper intake profile of microelements with an emphasis on the zinc-to-copper ratio. They also linked an imbalance of these elements to the development of cardiovascular disorders (95). Zinc affects cardiac differentiation (2), the regeneration of cardiac muscle, lipid peroxidation in cardiac tissue (16), and the outcome of surgical procedures on an open heart (93). In general, dietary zinc deprivation is lethal in mice, and in humans it is associated with severe disorders related to growth, maturation, and immunity. The complexity of zinc-dependent functions is further demonstrated by the fact that an excess of zinc is also associated with a number of abnormalities, the best illustrated in neuronal tissue (9, 42–44). Diabetic cardiomyopathy is associated with serum zinc deficiency (26), contradicting elevated intracellular zinc in cardiomyocytes (5). However, zinc supplementation via induction of cardiac metallothionein (MT) prevents development of diabetic cardiomyopathy in a rat model (110).

Zinc is critical for the structural integrity of the cells, since it influences membrane stability and cytoskeletal organization. Intracellular zinc homeostasis is challenged by oxidative stress, acidosis, and possibly by the signaling network, and it is an essential regulator of apoptosis. A specific protective role for a labile zinc pool against apoptosis has emerged within the last two decades (106). The limited regenerative potential of adult cardiac myocytes, along with the exciting possibilities of regeneration of cardiac muscle with cardiac progenitor cells, recognizes the necessity for new strategies. New knowledge would allow suppressing undesirable cell death in patients with ischemic heart disease, heart failure, myocarditis, arrhythmogenic right ventricular dysplasia, and immune rejection after

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cardiac transplantation (myocardial apoptosis has been recently reviewed in Refs. 1, 52, and 94).

The dynamic properties of zinc can be traced back in evolution to single cell organisms. Hence, under the conditions of nutritional imbalance, exchangeable zinc ions transiently accumulate in the vesicular compartment of the yeast *Saccharomyces cerevisiae* (21). A pool of cellular available zinc participates in phosphorylation/dephosphorylation cascades in mammalian cells, suggesting the existence of a signaling system based on zinc as second messenger. As a result, intracellular free zinc levels are strictly regulated by a complex system that includes transporters, storage vesicles, and buffering zinc-binding proteins, metallothioneins.

Zinc signals are often compared to the calcium signaling system, and even courageously Zn^{2+} was named "Calcium of the 21st century." Indeed, zinc and calcium are ubiquitous intracellular metals. Both are vital for cellular function. Both require tight control of their homeostasis. Similar to calcium, there is a mechanism for zinc sequestration into the storage compartment. It can be disputed whether a specific Ca^{2+}-sensing protein calmodulin is comparable to metallothionein. Although molecular mechanisms of metal regulation by the two proteins are different, the functional outcome is the same, specifically where the transfer of information from the ion to the effector protein is concerned. Yet, the principle of zinc signaling is based on different and unique chemical properties of interaction of zinc ions with redox reactive sulfur ligands.

Ironically, many of the tools that were originally designed for monitoring Ca signals can be used effectively for Zn^{2+} determination. Thus, the first report of intracellular Zn^{2+} concentration in cardiomyocytes is based on fura-2 measurements performed in the presence of a Zn^{2+} chelator (5). Ayaz and Turan estimated resting Zn^{2+} in cardiomyocytes to be 0.52 ± 0.06 n*M*. Remarkably, this level is nearly double in diabetic cardiomyocytes.

Since fura-2 or Mag-fura-2 initially used for Zn^{2+} determination were lacking specificity, making it difficult to separate zinc signals from calcium signals, the need for the development of zinc-specific probes has been growing with the expansion of zinc signals research. The number of newly developed Zn^{2+}-specific probes has been increasing exponentially since the late 1990s. These probes differ in their chemical properties and physical characteristics, and provide different benefits depending on experimental goals. For example, the Zinquin, the zinc probe that has a nanomolar affinity for zinc, overestimates the level of Zn^{2+} due to some degree of repartitioning of weakly bound zinc, the so-called labile zinc. On the other hand, the pool of labile zinc is possibly the most physiologically relevant and can be readily exchanged with an altered cellular state.

Whereas the total zinc concentration in eukaryotic cells is quite high (approximately 200 μ*M*), cytoplasmic free zinc concentrations are in the range of hundreds of picomolars. Thirty to 40% of the cellular zinc is localized in the nucleus, 50% in the cytosol and organelles, and the remainder is associated with membranes (107). Under physiological conditions, the majority of zinc within the cells is complexed by proteins. It took some time to realize that zinc indeed is not only a structural component of the proteins, but also an important regulatory ion. Intracellular zinc is tightly regulated and fluctuates dynamically.

An intense interest of the researchers to zinc has been stimulated by the discovery of a new regulatory function of reactive oxygen species (ROS), which can function as intracellular second messengers (19). Reactive oxygen species have been implicated in diverse cellular processes, including growth factor signal transduction, gene expression, and apoptosis. ROS are produced as a part of physiological signaling by hormones, lymphokines, and growth factors (20, 39, 114). There is growing evidence that many proteins are regulated by a redox environment through the reversible oxidation of their cysteine residues. The amino acid cysteine combines catalytic activity with an extensive redox chemistry and unique metal binding properties. Biologically zinc ions can act both as antioxidants and pro-oxidants. Both zinc deficiency and zinc overload elicit oxidative stress.

This paper presents an overview of the proteins regulating zinc homeostasis, as well as the molecular aspects of metal/redox regulation of protein function, with the accent on transcription factors and proteins of cytosolic signaling. The last part describes the hypothetical model of so-called protein "redox zinc switch," the redox sensing mechanism within the regulatory domain of protein kinase C (Fig. 1). Implications for cardiophysiology are discussed.

ZINC TRANSPORT AND STORAGE

Zinc transport

Tight control of cellular zinc homeostasis suggests the existence of zinc transporting systems as the first requirement, the way of elimination of excessive zinc to protect cell against zinc toxicity, and the routes for zinc entry to maintain zinc supplies for metabolic and regulatory purposes. New insights into mammalian zinc metabolism have been acquired through the identification and characterization of zinc transporters. The understanding of zinc transport at the molecular level began with the first functional evidence of zinc transport activity by ZnT-1 (90, 91). Rapid progress has been made since then, leading to the discovery of the proteins encoded by two solute-linked carrier (SLC) gene families: ZnT (SLC30) and Zip (SLC39). There are 10 ZnT and 15 Zip transporters in human cells (77). They appear to have opposite effects on Zn^{2+} homeostasis: ZnT transporters facilitate zinc efflux from cytoplasm either into various intracellular compartments (81), or across plasma membranes. Zip transporters promote extracellular zinc uptake and vesicular zinc release (Fig. 2). A common feature of ZnT transporters is a number of highly conserved histidine residues, which seem to be important for metal ion recognition and transport (77). Both transporter families exhibit tissue-specific expression and differential regulation by zinc deficiency and excess, and by signaling cascades (Fig. 2).

The ZnTs are a growing family of proteins involved in sequestration of intracellular zinc. ZnT-1, the first mammalian transporter discovered, has the simplest gene structure (90). With two exons, this gene predicts a 507 amino acid protein with six transmembrane domains with both N- and C-termini on the cytoplasmic side of the membrane. Like other ZnT proteins, ZnT1 has a long histidine-rich loop, which could represent a metal-binding domain. It is possible that ZnT proteins function as dimmers or multimers. ZnT-1 is the most

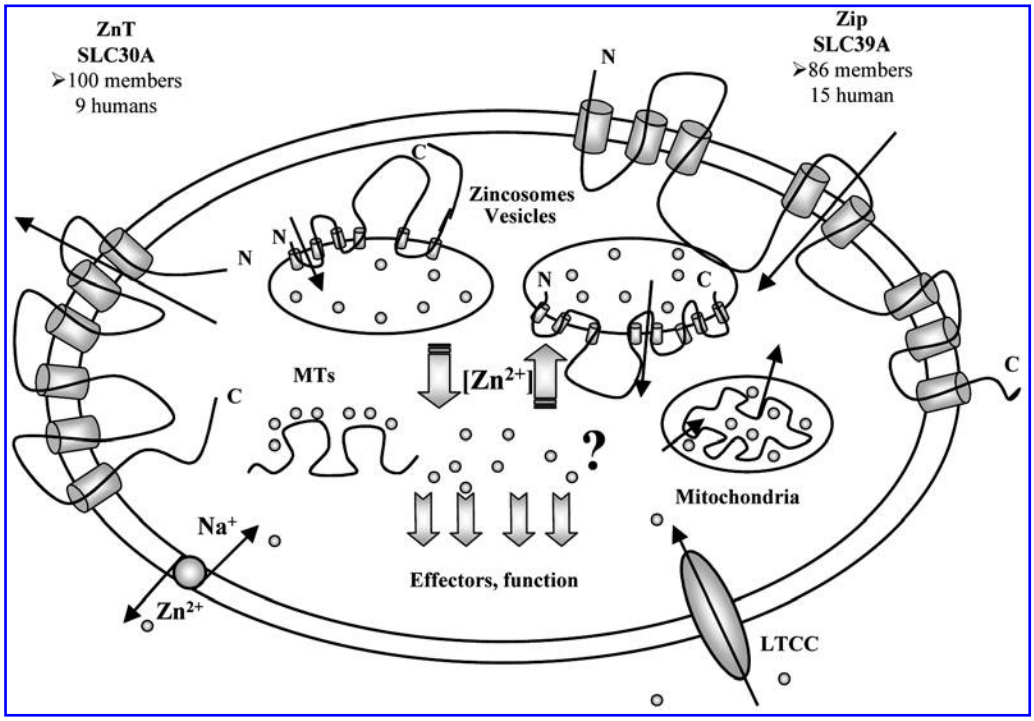

FIG. 1. Molecular partners of zinc homeostasis. Zn^{2+} efflux is mediated by ZnT-1 transporter, the member of SLC30 gene family. Other ZnT proteins facilitate zinc sequestration by cytosolic vesicles: zincosomes. Sodium/zinc exchange is another mechanism for intracellular zinc extrusion. On the other hand, Zip proteins, SLC39 family members promote extracellular zinc uptake and zinc release by zincosomes. The major route of zinc entry into most cells is L-type calcium channel (LTCC). Mitochondria and zincosomes are storage compartments for excessive zinc and potential regulators of zinc-based signaling. Metallothioneins are protein-based storage sites and zinc donors to various proteins. Liberated or labile zinc regulate thousands of known and unknown proteins and as a result influence physiological responses. The transporter proteins are oversized to emphasize the structural features.

ubiquitously expressed ZnT protein. The well-documented cellular role of ZnT-1 is protection against zinc toxicity (77).

Expression of ZnT-1 is regulated in a tissue-specific manner by the level of available zinc. Zinc deficiency leads to a significant decrease in mRNA content, while zinc supplementation induces marked increase in the mRNA level (76). The molecular mechanism for ZnT-1 expression is not delineated but it appears to be identical to that of metallothionein gene (discussed further in this review), specifically the involvement of metal response element (MRE) in the ZnT-1 promoter and activation of transcription through metal responsive transcription factor (MTF) (74).

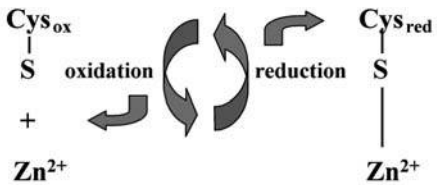

FIG. 2. Redox chemistry of the zinc–cysteine interaction. Oxidation and reduction of protein cysteines are linked to the release and binding of the zinc ion, respectively. Thiols can be oxidized reversibly to disulfides and cysteinesulfenic acid. Oxidation of cysteines to cysteinesulfinic and cysteinesulfonic acids is irreversible. Zinc is in turn a potential modulator of cysteine reactivity. This interdependent interaction forms a chemical basis for a protein "redox zinc switch."

Although it was discovered several years ago, it is unclear how ZnT-1 modulates zinc fluxes; it has been recently suggested that the protein may be involved in the regulation of zinc influx rather than efflux. Peculiarly, the major route of zinc entry into most cells is L-type calcium channel (LTCC) (4). When LTCC was co-expressed with the ZnT-1, the rate of Zn^{2+} influx reduced threefold (97).

Although transition metals are generally viewed as Ca^{2+} channel blockers, single channel recordings suggest that zinc can enter the myocytes through dihydropyridine-sensitive calcium channel. Therefore, activation of LTCC permeability for Zn^{2+} is the primary machinery of voltage-mediated gene expression (4). Despite the general important physiological consequences of Zn^{2+} influx, the mechanism of LTCC permeation for Zn^{2+} remains elusive. In mouse cortical neurons, an inward current detectable at micromolar doses of zinc was sensitive to a blockade by Cd^{3+} and nimodipine. Channel amplitude and voltage sensitivity depended upon doses of Zn^{2+}. Zinc ions could permeate calcium channels in the presence of Ca^{2+}. Currents recorded with Zn^{2+} were unaffected by up to equimolar calcium concentrations. Zn^{2+} current mediated by voltage-gated calcium channels was enhanced by extracellular acidity, while Ca current was inhibited by lowering pH (62).

The co-expression experiments suggest a regulatory role for ZnT-1 in permeation of the LTCC for zinc ions. ZnT-1 does not affect LTCC expression level, indicating that this regulation is based on functional interaction between the two

proteins or between ZnT-1 and LTCC regulator. It should be noted that ZnT-1 is also a regulator of Ca responses. Ca entry via LTTC was diminished with ZnT-1 overexpression. In line with this negative control, capacitive Ca entry is inhibited by ZnT-1. This inhibition is likely to occur through interaction of ZnT-1 with the redox-sensitive extracellular site of store-operated Ca channel (42). Similarly, zinc itself has a negative impact on capacitive Ca entry by affecting extracellular thiols, as shown in mouse astrocytes (71). In addition, one more route of cross communication of Ca^{2+} and Zn^{2+} homeostasis is negative regulation by zinc ions of ryanodine binding to sarcoplasmic reticulum that leads to inhibition of Ca^{2+} release in both skeletal muscle and cardiomyocytes (5).

ZnT-1 is the only mammalian zinc transporter acting as a cellular zinc exporter. All other ZnTs participate in sequestration of zinc ions into endosomes, secretory granules, synaptic vesicles, Golgi apparatus, or trans-Golgi network (77). They are unlikely to share the same channel-regulating activity. The detailed mechanism of ZnT-mediated zinc sequestration remains to be established. Recently discovered ZnT-5 is of particular interest. This vesicular transporter was reported almost simultaneously by two groups (17, 58). Unlike many other transporters, it has 12 [15, according to Kambe *et al*. (58)] transmembrane spanning domains. The protein expression is also regulated by high zinc and possibly mediated by MTF1. ZnT-5 has unusual although uncharacterized functions within the cardiovascular system. Intriguingly, male ZnT-5 null mice develop fatal arrhythmias and die of heart block and sinus bradycardia at 15 weeks of age (50). It should be noted that independent of ZnT proteins, recently discovered sodium/zinc exchange mediates extrusion of Zn^{2+} from mammalian cells. The exchange was reversible upon reversal of sodium gradient, and energy independent, consistent with a mechanism involving a secondary active transporter (85).

Zip proteins (24) have been divided into four subfamilies: I, II gufA, and the LIV-1 subfamily of ZIP transporters (LZT). Most Zip proteins have eight transmembrane domains with extracellular or intravesicular amino- and carboxy-termini. Interestingly, the LZT subfamily members have a long extracellular N terminus with 2 to 34 histidines and the novel metalloprotease motif (HEXPHE), which could suggest alternative functions (104). As ZnTs, Zip transporters are energy independent. Their expression is stimulated by elevated Zn^{2+} and may be linked to oxidative metabolism (28). LIV-1 expression is stimulated by insulin, IGF-1, TGFα, and EGF (25). Oxidative treatment of C6 glioma cells resulted in 50% of total zinc exported through the plasma membrane (44). Our study shows that stress imposed by ischemia and reperfusion (I/R) leads to zinc depletion of cardiac tissue. Thus, understanding the mechanism of export is crucial in cardiac cells. Although, the research on Zip family proteins shows rapid progress, no convincing data exist on their function in heart, providing a fertile field for future investigations.

Sequestration of zinc by mitochondria

Sequestration of zinc ions into intracellular vesicles by ZnTs is not only the way of cell protection from Zn^{2+} overload. It also represents the machinery of Zn^{2+} storage. In most cell types, zinc-containing vesicles, so-called "zincosomes," are observed under normal conditions (28). In our experiments using neonatal cardiomyocytes, the morphological appearance of *N*-(6-methoxy-8-quinolyl)-p-toluensulfonamide (TSQ) staining changed from homogeneous cytoplasmic distribution of fluorescence in control toward a patchy distribution in phorbol-myristate-acetate (PMA)- or hydrogen peroxide- treated cells, reminiscent of the vesicle-associated zinc accumulation seen in other cells. In adult rat cardiomyocytes, an increase in TSQ fluorescence was first noticeable only at the cell periphery, then spread all over the cell forming typical vesicular structures (Korichneva *et al*., unpublished observations).

In addition to zincosomes, the mitochondria are now a well-established compartment taking up elevated cytosolic zinc. For the last decade, mitochondria have been known as key stations in apoptotic signaling. Myocardial cell apoptosis contributes to cardiac dysfunction, including ischemia/reperfusion injury. It has been demonstrated that components of ischemia activate the mitochondrial death pathway in cardiac myocytes. This pathway involves depolarization of the mitochondrial membrane and opening of the permeability transition pore (PTP), a channel through which molecules ≤1.5 kDa pass. Release of apoptosis-inducing factor, Smac, procaspase, and cytochrome C following membrane rupture initiates the execution process, and seals the fate of the cells. Mitochondria are firmly integrated into the cellular control circuits. The afferent signals affecting mitochondrial function are not fully understood. Among others, they appear to include ROS as redox triggers of mitochondria and activators of a number of upstream signaling molecules that are recruited to mitochondria. The subsequent generation of ROS by mitochondria may constitute a feed-forward loop to monitor the redox microenvironment and to initiate appropriate responses. While cytosolic redox triggers lead to zinc release, the equilibrium in zinc compartmentalization shifts in such a way that zinc ions are quickly taken up by mitochondria as a consequence of redox stimulation. A detailed study by Sensi and colleagues (99) clearly demonstrates opposite roles of physiological and excessive zinc on mitochondrial function. Submicromolar levels, comparable to those that might occur during strong mobilization from intracellular compartments, induce membrane depolarization, increase currents across the mitochondrial inner membrane as detected by direct patch clamp recording of mitoplasts, increase oxygen consumption, and decrease ROS generation. However, high doses of zinc decrease oxygen consumption and increased ROS generation. Therefore, excessive mitochondrial Zn^{2+} sequestration would lead to a marked dysfunction of these organelles, characterized by prolonged ROS generation (96–99). While most of the studies of zinc function in mitochondria were performed on brain cells or tissue, or even mitochondria isolated from brain, we observed the relocation of zinc pool in mitochondria of cardiac cells upon stimulation (Korichneva *et al*., unpublished observations).

The mechanisms of zinc-induced mitochondrial dysfunction are not delineated. Several molecular targets have been identified. Zn^{2+} inhibited alpha-ketoglutarate-stimulated mitochondrial respiration and alpha-ketoglutarate dehydrogenase complex isolated from the pig heart (10). Zn^{2+} similarly to Ca^{2+}, both at 10 μ*M,* triggered mitochondrial permeability transition, induced cytochrome C release and ROS production by

isolated liver mitochondria (9). Zinc in cardiac mitochondria reversibly inhibited the bc1 complex. Existence of a mitochondrial target for Zn^{2+} upstream of the bc1 complex has been also suggested. Zinc is shown to be an efficient inhibitor of mitochondrial cytochrome C oxidase activity rapidly interacting with the enzyme at a site exposed to the aqueous phase corresponding to the mitochondrial matrix (73). On the whole, tissue specificity would define the level of toxic doses for zinc ions.

Zinc, taken up by mitochondria, can in turn be released in a Ca-dependent manner. At physiological pH, much of the zinc released from mitochondria is rapidly buffered by the cytosolic proteins, thus preventing large increases in Zn^{2+}.

Zinc storage proteins metallothioneins

The metallothioneins (MT) deserve a special place in the area of zinc homeostasis due to their unique role as protein-based storage sites for zinc. MTs localize in the intermembrane space of mitochondria, the cytosol, and the nuclear. Thirteen forms of human MTs originate from different genes. MTs are rather small, cysteine-rich heavy metal binding proteins that have the most complex Zn^{2+}-Cys coordination of all zinc containing proteins known (27, 87, 102). One third of all amino acid residues of MTs are strictly conserved cysteines. They bind seven zinc ions in zinc–thiolate clusters. Despite the fact that zinc binding to MT has the highest affinity compared to other proteins (-log *K*d equals ~13), zinc in MT is readily exchangeable (56). Experimental data suggests that the likely way the MTs exchange zinc is by direct interaction with other zinc ligands (59). These properties designate to MTs two major antistress functions: in addition to playing a central role in heavy metal detoxification, these proteins are capable of protecting the cells from oxidative stress. The latter may be exerted due to chaperone-like activities of MTs that are capable of donating zinc to target metalloproteins, which in turn could have lost zinc under stress conditions. Studies by Kang *et al*. (60), using a cardiac-specific MT-overexpressing transgenic mouse model, have demonstrated that MT inhibits myocardial injuries triggered by oxidative stress. Myocardial oxidative stress has long been known to play a major role in several pathophysiological responses, including ischemia/reperfusion injury, cardiotoxicity induced by environmental chemicals and therapeutic drugs, and heart failure resulting from multifactorial manifestations. Under acute or chronic oxidative stress, ischemia/reperfusion, doxorubicine treatment, or dietary copper restrictions, MT-overexpressing mouse hearts revealed marked resistance to damage based on biochemical and functional analysis. Clearly, ischemia/reperfusion-induced myocardial injury was inhibited by MT via suppressing apoptosis (60). MTs inhibited mitochondrial cytochrome C release and caspase 3 activation. The protective role of MT in myocardial I/R may be accomplished via zinc release in oxidized environment, followed by the transfer to zinc binding proteins necessary to support cell function in the conditions of oxidative stress. Accordingly, reactivity of sulfur ligand is the key to the mechanism of action of MTs (79). MT does not release all of its seven zinc ions except under the conditions of severe oxidative stress when modifications of thiols in MT zinc clusters occur. It contains at least one that is prone to be released than the others, and it originates from the three-zinc cluster (57). It has now been demonstrated by direct thiol modifications that disulfides are present in MT from MT-overexpressing transgenic mouse hearts and they are increased under the conditions of oxidative stress (31).

The understanding of MT function was significantly advanced by the discovery of the apoprotein, thionein (T). It was found that T is present in the tissues in the amounts comparable to those of MT, underscoring the dynamics of zinc distribution in the MT/T pair (112). Binding of zinc to T is highly cooperative, and the MT/T ratio (but not MT on its own) correlates with the available cellular zinc (53). Different tissues have different MT/T ratios, suggesting that changes in this ratio affect availability of zinc in the cells. Zinc transfer can occur in either direction, from MT to proteins or from proteins to T. The direction of zinc transfer reactions would depend on the redox status of the cells. It has been shown that T rapidly activates a group of enzymes in which zinc is bound at an inhibitory site (78).

Translocation of MT was observed between cytosol and nucleus, cytosol and mitochondria, as well as through plasma membrane. Thus, MT participates in compartmentalization of available zinc within the cell. Import of MT into mitochondria inhibits respiration. The electron transfer chain is identified as a primary site of inhibition. MT inhibition involves zinc delivery to mitochondria (112). The transported apoform of MT, thionein, activates zinc-inhibited respiration with a 1:1 stoichiometry. It should be noted, however, that inhibition of respiration by MT was demonstrated in the liver but was not confirmed in the heart.

A critical physiological function of MTs that has been suggested is to control Zn^{2+} availability to proteins requiring Zn^{2+} for activity. The biochemical proof for the transfer was demonstrated recently in heart extracts from MT-null mice incubated with ^{65}ZnMT. Myocardial proteins receiving ^{65}Zn were separated electrophoretically. The analysis of a unique band revealed mitochondrial aconitase among the proteins accepting MT bound zinc. The mitochondrial but not cytosolic aconitase was immunoprecipitated with MT, confirming direct interaction (30).

Mammalian metallothionein genes are transcriptionally regulated by heavy metals through metal response elements (MREs). The MRE-binding transcription factor-1 (MTF-1) is a protein containing six C(2)H(2)-type zinc fingers. An intact zinc finger domain is required for metal-induced recruitment of MTF-1 to the MT-I promoter (55). Mutational analysis showed that the six zinc fingers are not functionally equivalent, sharing distinct roles such as direct DNA recognition and regulatory function (65). The transcription of four known MT genes is rapidly upregulated in response to high zinc or oxidative stress. DNA-binding activity of MTF-1 *in vivo* and *in vitro* was reversibly activated by zinc interaction with the zinc finger domain controlled by redox conditions. Accordingly, MTF-1 serves one of the sensors of free zinc alterations in the cell.

As mentioned earlier, ZnT-1 transporter transcription is initiated by MRE-binding to MTF-1. However, regulations of expression of ZnT-1 and MT by zinc appear to be independent processes. Transgenic mice with a null mutation in the

MT gene, and transgenic mice overexpressing the protein, exhibit normal ZnT-1 expression.

Another type of intracellular redox stress, nitrosative stress and its relation to zinc homeostasis is receiving increasing attention. Both chemical compounds that generate NO, and an endogenous source, inducible nitric oxide synthase-derived NO, trigger nuclear MT translocation and intracellular Zn^{2+} release (62). NO stress increases mRNA expression of both MT-1 and MT-2, presumably via Zn^{2+} release, in murine aortic endothelial cells. The transient nature of this phenomenon witnesses the signaling function of zinc ions, suggesting that when stress has declined, modified zinc fingers are restored to be prepared for response to the next message.

ZINC-MEDIATED REGULATION OF CELLULAR FUNCTIONS

Protein regulation

There are thousands of zinc-containing proteins in the human proteome. This review highlights a few examples of zinc involvement in regulating the function of these proteins. In support of crosstalk of zinc and redox signals, antioxidant enzymes are the first example of zinc regulation. Copper–zinc superoxide dismutase (CuZnSOD) is an antioxidative defense enzyme that attenuates myocardial ischemia/reperfusion injury. The structural interplay of conserved disulfide bond and metal-site occupancy in human SOD1 is of increasing interest, as these posttranslational modifications are known to dramatically alter the catalytic chemistry, subcellular localization, and susceptibility of the protein to aggregation. Either the addition of Zn^{2+}, or the formation of the disulfide, leads to a shift in equilibrium that favors the dimeric species, the mature form (3).

SAG/ROC/Rbx/Hrt, a zinc-containing RING (really interesting new gene) finger proteins with characteristic C3HC4 or C3H2C3 motifs appear to act as E3 ubiquitin ligases and play important role in many vital processes in myocardium. In humans, both SAG and ROC1 are ubiquitously expressed at a very high level in heart and skeletal muscle. SAG is protective against apoptosis (103). Another group of proteins containing a zinc-binding fold, BIR (baculoviral IAP repeat)-containing proteins are inhibitors of cell death and act by binding to active caspases (101).

The DNA repair enzyme, polyADP-ribose polymerase (PARP) is a zinc-dependent enzyme that is activated by binding to DNA breaks. Poly(ADP-ribosyl)ation of nuclear proteins by PARP converts DNA damage into intracellular signals that activate either DNA repair by the base-excision pathway or cell death. As a result, PARP zinc finger modules found at the N-terminus were initially described as nick sensors. The specific hypothesis that zinc toxicity might be partly mediated by PARP has been tested. PARP was activated in cultured mouse cortical astrocytes after a toxic acute Zn^{2+} exposure (350 μM Zn^{2+} for 15 min). The neurotoxicity induced by acute, but not chronic, Zn^{2+} exposure was reduced in mixed neuronal–glial cultures prepared from mutant mice lacking the PARP gene. Additionally, an acute influx of labile Zn^{2+} induced apoptosis via activation of caspase in human leukemia HL-60 cells (66). The area of zinc regulation of proteins, classical players of apoptotic pathway, is thus far underexploited in cardiac tissue. The sole evidence of blocking caspase-3 activation by zinc chloride in cardiac allografts suggested that there may be synergistic effect of zinc ions and cyclosporine A in decrease of apoptosis (70).

There is more evidence associated with zinc regulation of cardiac matrix metalloproteinases (MMPs). MMPs are zinc-dependent endopeptidases that are traditionally known for their role in extracellular matrix degradation remodeling in physiological and pathological processes, including ischemia, myocardial infarction, and cardiomyopathy. Catalytic sites are preserved in the zymogene form by $ZnHis_3Cys$ coordination. Upon increased oxidative or nitrosative stress, a catalytically active enzyme is released via a "cysteine zinc switch" mechanism in which cysteines can be oxidized with formation of a disulfide S-oxide and zinc ion of the active site is dissociated from a cysteine residue, allowing the enzyme to function (43, 83). In the cardiovascular system, a prevalent MMP, MMP-2, can degrade such extracellular matrix proteins as collagens, fibronectin, elastic, and laminin. Loss of collagen leads to progressive contractile dysfunction (13, 88). Recent studies reveal a growing number of nonmatrix substrates: endothelin-1 yielding a novel vasoconstrictor (32), or the vasodilator calcitonin gene-related peptide (33). Studies by Schultz and colleagues uncover a novel biological role for MMPs by showing the presence of MMP-2 within myofilaments and in the nucleus, where the peptidase was able to cleave troponin I and PARP, respectively (38, 109). These biochemical events would contribute significantly to impairment of myocardial recovery in postischemic reperfusion or at other conditions of oxidative stress. Indeed, it has been shown on isolated perfused rat hearts that peroxynitrite-induced myocardial injury is mediated by MMP-2 (108). The proteolytic cleavage of myosin light chain and troponin I by MMP-2 was clearly one of the reasons for myocardial ischemia injury (96).

Effects of zinc on gene expression

Many cardiac abnormalities that are induced by zinc deficiency are associated with alterations in the expression of genes that encode myocyte structural proteins and the proteins involved in signal transduction, redox responses, and growth and energy utilization. The discovery of so-called zinc fingers in the early 1980s gave a start to a series of innovations related to functional implications of zinc–sulfur ligand interactions. Since 3% of the whole human genome encode proteins with zinc fingers, the significance of physiological consequences of zinc and cysteine mutual regulation is difficult to underestimate (51). Zinc in zinc fingers can be coordinated either by two cysteines and two histidines ($ZnCys_2His_2$), three cysteines and one histidine ($ZnCys_3His$), or cysteines only ($ZnCys_4$). The chemistry of this interaction (*e.g.*, tight zinc binding to sulfur ligand along with redox active coordination for zinc ion in zinc fingers) allows these domains to confer multiple activities to the proteins, such as control of conformation and function, translocation, and sensing zinc concentration. Evidence exist that a disruption of thiols in zinc–sulfur clusters leads to reversible inhibition of zinc finger structures and DNA binding, providing molecular mecha-

nisms to regulate gene transcription via modulation of zinc–thiol interaction (72). A few examples will illustrate how zinc and redox signals converge on zinc fingers of transcription factors.

The transcription factor p53 controls the proliferation and survival of cells exposed to DNA damage. Targeted deletion of p53 prevents cardiac rupture after myocardial infarction in mice (80). The specific DNA-binding domain of p53 (residues 102–292) has a complex tertiary structure that is stabilized by zinc. It has been shown that the exposure of cultured cells to the membrane-permeable zinc chelator N,N,N', N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) induces wild-type p53 to accumulate in an immunologically "mutant" form (PAb240+, PAb1620-) with decreased DNA-binding activity. Removal of TPEN from culture medium allowed p53 to refold into the immunologically wild-type form, followed by a transient increase in DNA binding, expression of the cyclin-dependent kinase inhibitor p21WAF1, and cell-cycle delay in the G1 phase. Thus, modulation of intracellular zinc induced conformational changes in p53 that activated wild-type function, suggesting that metalloregulation may play a role in controlling p53. This redox and metal sensitivity may be one of the biochemical mechanisms by which p53 acts as a "sensor" of multiple forms of stress. The involvement of thioredoxin, redox factor 1, and metallothionein in control of p53 protein conformation and activity has been discussed (46).

Retinoic acid-mediated gene expression is essential for cardiovascular morphogenesis. DNA-binding domain of nuclear retinoic acid receptor contains two zinc fingers with zinc tetrahedrally coordinated by four cysteines. Under oxidative stress conditions, DNA binding of zinc finger is abolished and zinc is released. The effect is reversible: the combination of reduced glutathione and Zn^{2+} restores activity. The Early Growth Response protein (Egr-1) is a classical C(2)H(2)-zinc-containing transcriptional regulator involved in the control of cell proliferation and apoptosis. Its DNA binding activity is redox regulated *in vitro* via oxidation/reduction of Cys residues (92).

The cardiac-restricted zinc finger transcriptional factor GATA-4 has been implicated as a critical regulator of inducible cardiac gene expression and as a potent mediator of the hypertrophic program. By interacting with NFATc in endothelin-1 treated cardiomyocytes, GATA-4 mediates protection against oxidant-induced apoptosis (23). GATA-4 induces transcription of survival factors, including anti-apoptotic molecule bcl-2 in cardiac tissue. Zinc deficiency induced cardiac abnormalities associated with alterations in the expression of genes regulated by GATA-4. Thus, two genes critical for heart development, α-myosin heavy chain and cardiac troponin I, were downregulated in Zn-deficient rat fetuses. (23). It has also been suggested that GATA-4 DNA binding is upregulated by a mechanical stretch in the isolated rat atria via p38 and extracellular signal-regulated protein kinase (105).

Zinc control of cytosolic signaling

Zinc deficiency may indirectly lead to the activation of transcription factors that do not possess zinc finger domains in their structure. Thus, oxidant-sensitive transcription factors NF-κB and AP-1 are generally activated in zinc deficiency, which occurs possibly as a result of oxidative stress followed by activation of p38 and JNK (86).

The work of Keen and colleagues contributed significantly to our understanding of the effects of zinc deficiency on both transcription factors and signaling kinases (15). In general, zinc deficiency is characterized by attenuation of growth factor signaling pathways and an amplification of p53 pathways. This outcome is achieved by hypophosphorylation of anti-apoptotic kinases, AKT and ERK, under the conditions of zinc deficiency, and activation of p38 and JNK. It is not clear, however, to what extent the production of ROS is involved in these effects and whether zinc may have direct regulatory function on the enzymes. It could be also important to understand time-dependency of zinc regulation of signaling kinases. The complexity of zinc effects on MAP kinases contributes to controversy between the *in vitro* and *in vivo* studies. As such, opposite to zinc deficiency, zinc chelation inhibits activation of p38. Tissue specificity and doses studied are other crucial factors playing a part in the controversy. How zinc affects MAPK function in cardiac tissue is still an open question.

As another group of regulators of protein phosphorylation, protein phosphatases are also subject to zinc control. Zinc is an effector of insulin/IGF signaling. Tight inhibition of PTPs is likely responsible for insulinomimetic properties of zinc ions. The study by Haase and Maret established that zinc inhibits protein tyrosine phosphatases (PTPs) as a cause for these effects (45). *In vitro* inhibition of PTPs occurs at nanomolar zinc concentrations. Incubation of cultured cells with zinc and zinc ionophore pyrithione augments tyrosine protein phosphorylation, while chelation of intracellular zinc with TPEN suppresses insulin- and IGF-1-stimulated tyrosine phosphorylation.

The principle of signal transduction and definition of a second messenger first evolved from the discovery of hormone-regulated adenylate cyclase, an enzyme that converts the signal from G-protein coupled receptor to synthesis of cAMP. Since then, multiple consequences of cAMP-dependent signaling have been investigated in detail, including regulation by hormones, α and βγ subunits of G-proteins, feedback phosphorylation by associated kinases, control by ionic environment, and plasma membrane lipids. Recent interest to zinc signals provoked analysis of its effects on membrane associated and soluble adenylate cyclase. It has been shown that zinc inhibits hormone and forskolin stimulation of cAMP synthesis in N18TG2 cells. Crystallographic data of the truncated soluble AC suggest a possible mechanism by which zinc could inhibit AC activity. It turned out that micromolar concentrations of Zn^{2+} bound to a site different from Mg^{2+} binding sites involved in catalysis. Using extrinsic and intrinsic fluorescent measurements, the authors showed a conformational response of the enzyme to zinc binding (63). Zinc deficiency and altered myocardial adenylate activity commonly occur in diabetes with hormonal-stimulated enzymatic activity, which is significantly lower in diabetic rats on a low zinc diet (82).

Zinc has also been shown to influence cAMP signaling by affecting cyclic nucleotide phosphodiesterases (PDE). After first observations of an inhibition of cAMP and cGMP hydrolysis by zinc in bovine heart, there have been several reports of both activating and inhibitory effects of zinc on different PDE subfamilies. For example, cGMP specific PDE V

is activated by zinc at submicromolar levels and inhibited at higher doses of zinc (37).

As a separate issue, it is worth noting the so-called "zinc sensing receptor" role in the modulation of classical signaling pathways. Extracellular zinc may not only mediate physiological responses by entering the cells but also by stimulating a zinc-sensing receptor (ZnR) on the surface of some cell types. This receptor, though not yet cloned, appears to be coupled to phospholipase C via pertussis toxin sensitive G-protein and linked to intracellular Ca release and MAP kinase pathway activation (48). Desensitization of ZnR by high doses of zinc is followed by approximately 90% inhibition of Zn^{2+}-dependent ERK1/2 phosphorylation. Physiological importance of ZnR activation was supported by robust activation of Na/H exchanger in colon epithelial cells, which was blocked by MAPK pathway inhibitor U0126 (6). These observations are in line with several others that show that treatment of cells with $ZnCl_2$ increases phosphorylation of ERKs (47). The apparent affinity of the putative receptor is estimated to be close to 80 μ*M*. A critical related question would be whether zinc-sensing receptor is functional *in vivo* given the extracellular zinc level of 10–20 μ*M*. In certain pathological conditions, such as brain ischemia, zinc is massively released from synaptic vesicles into the extracellular space. Consequently, elevated zinc can activate the receptors of postsynaptic membranes. If the zinc-sensing receptor exists on cardiomyocytes, its activation during myocardial ischemia/reperfusion would have significant physiological impact. In fact, since we have suggested that zinc is extruded from the cells under oxidative stress in myocardium (68), zinc ions could play a role of autocrine regulator providing feedback loop controlling cardiac responses.

"REDOX ZINC SWITCH" HYPOTHESIS

Redox chemistry of zinc interactions

Despite skepticism that the strongly reducing intracellular environment would permit significant oxidation of cysteine residues within zinc finger proteins, there is compelling evidence that oxidation occurs both *in vivo* and *in vitro*. The reports demonstrating reversible oxidation of zinc-coordinated cysteines with loss of zinc binding function *in vitro* were shown to reflect accurately the changes in intact cells, and these in turn have been linked to physiological changes. A critical biological partnership of zinc and sulfur ligand is reviewed by Maret (78).

Chemical flexibility of transition metal allows zinc to impose conformational changes on the proteins it binds (75). Being redox inert, zinc plays a part in electron transfer complex shifting protein electron density. In proteins, zinc can bind mainly to cysteine, histidine, aspartate, and glutamate residues. Cysteines possess the unique structural features that allow them to combine metal binding properties with catalytic activity and extensive redox chemistry. Within the zinc coordination center, zinc ions potentially can modulate reactivity of cysteine thiol groups towards oxidation. Interdependency of these three aspects permits the redox regulation of proteins, metal control of redox activity, and redox control of metal-based catalysis. The most important and extraordinary property of zinc–sulfur ligand interaction is release of zinc under oxidative environment, converging the redox signaling and zinc metabolism (Fig. 1). Cysteine-rich zinc binding protein domains are therefore able to act as "redox zinc switches' to sense the concentrations of both zinc and oxidants. Therefore, unlike thiol oxidation/reduction, "redox zinc switches" are controlled by zinc availability. Remarkably, until recently it was assumed that the redox inert zinc protects thiolates from oxidation. However, the recent studies suggest that a strong binding of zinc enhances the redox sensitivity of thiolates (79).

The unsurpassed study of the mechanism of the "redox zinc switch" operation was performed in a relatively simple cell model of the *Escherichia coli* chaperone bacterial chaperon Hsp-33. Hsp33 contains a C-terminal zinc finger domain that modulates activity by a redox-regulated, reversible hinge (8, 54). In an oxidizing microenvironment, thiols were converted to disulfide, zinc became uncoupled, and the unfolding of the protein led to the active enzyme. The reduced form in the presence of zinc was inactive. The solution structure of a recombinant 61–residue protein containing zinc-binding domain of Hsp33 suggests that loss of the bound zinc ions disrupts the well-folded structure, allowing the ligand cysteine residues to be oxidized (8). This finding implies that the redox response of zinc–cysteine clusters is biphasic and emphasizes a separate role for zinc in redox control mechanism. Our laboratory demonstrated that similar redox zinc switch operates the activation of protein kinase C (PKC), and perhaps other serine/threonine kinases containing homologous zinc finger domains (69).

It should be noted that in some instances the thiols of different polypeptides are oxidized under stressed conditions. For example, in nitric oxide synthase, the zinc site links two protomers, oxidation releases zinc and uncouples the enzyme. Ischemic conditions create more complexity on the redox switches. As such, the equilibrium of free versus chelated zinc is sensitive to environmental factors, in particularly to acidosis. Acidification appears to rapidly destabilize zinc–thiol binding, shifting equilibrium towards free Zn^{2+}. Taken together, chemistry of zinc–sulfur ligand interactions suggests that zinc homeostasis may play a very significant role in ischemic damage of tissues, including myocardium.

Zinc hypothesis of PKC activation: operation of the mammalian "redox zinc switch"

Perhaps a better understanding of the regulation of signaling kinases by zinc and redox changes is accomplished in the studies of PKC isoforms. PKC has always been the focus of increased interest due to the key role of this enzyme in signal transduction (111). Different isoforms translocate to different compartments to close proximity to substrates and perform multiple cellular functions. Like other Ser/Thr kinases, PKC is sensitive to variations of zinc concentration. More than 10 years ago, Forbes and Zalewski demonstrated that micromolar concentrations of zinc chloride induced specific association of PKC with the plasma membrane or cytoskeleton (34–36). The attachment of protein kinase C to the cytoskeleton was accompanied by an enhanced expression of binding sites for 3H-phorbol ester, a regulatory ligand of protein kinase C. The heavy-metal chelating agent 1,10-phenanthroline com-

pletely reversed the increased [3H]PDBu binding in cells pretreated with ^{65}Zn and ionophore, which was associated with a decline of about 20% in cell-associated ^{65}Zn, suggesting that a relatively small pool of intracellular Zn^{2+} acts on PKC. The authors suggested that this could be a membrane-associated pool. The active factor in the cytoskeleton was labile to protease, suggesting that protein kinase C binds to a cytoskeletal protein. PKC bound to a Zn^{2+} affinity column and was eluted by a metal chelator, confirming that Zn^{2+} interacts directly with PKC. The authors further proposed that putative binding sites for zinc were present in the regulatory domain of protein kinase C; however, no distinct role for zinc was obvious at that time.

Later, zinc was shown to activate PKC in neurons and platelets (71), and was required for cellular response mediated by PKC in lymphocytes. Timely studies by Keen and colleagues of the effects of zinc depletion on function of kinases revealed that decreased zinc lowered classical PKC activity and led to proteolysis of the novel PKC family member, PKCδ, to its 40 kDa fragment, a positive regulator of apoptosis via caspase-3 activation (14). This effect of zinc deficiency could be a direct impact on the PKC molecule, or it may be executed via increased oxidative stress, possibly by influence on copper-mediated ROS production.

The ability of PKC to regulate many cardiovascular functions is supported by the facts that many cardiovasotropic growth factors, such as angiotensin, endothelin, vascular endothelial growth, and permeability factor target PKC (12, 18, 22, 29, 84). The physiological importance of PKC can be surmised by the existence of multiple isoforms, which are usually arranged into groups according to their structure and cofactor requirements. Conventional PKCs (α, $\beta_{1/2}$, and γ) are Ca^{2+} dependent and activated by diacylglycerol (DAG); novel PKCs (δ, ε, η, and θ) are Ca^{2+} independent but activated by DAG; atypical PKCs (ζ and i/λ) are Ca^{2+} and DAG independent (12, 61). It has been shown that restricted physiological functions are limited to specific isoforms. Thus, cardiac PKCδ is activated by ischemia, and PKCe is involved in ischemic preconditioning, linking PKC function to redox control (7, 12, 16). Supplementation of perfusion solution with δ-specific RACK-derived peptide inhibitor confers protection of myocardium against ischemic injury.

Independent of the classical lipid-mediated pathway, PKC is also controlled by a redox mechanism. PKCs embrace two independent target sequences susceptible to oxidative modification (41). The two domains of PKC respond differently to redox changes. Thus, oxidation of thiols in the catalytic domains blocks enzymatic kinase function. More interestingly, oxidation within the regulatory sequence converts the protein to the catalytically competent form, while reduction reverses this process (40, 49, 64, 66). Physiologically, it would mean that the regulatory domain should be sensitive to lower doses of oxidants. Another manner in which such preferential oxidation might occur is through the facilitation of electron transfer by retinoids. It has been found that the redox activation of the kinase requires cofactors, retinol or its metabolites, since the high affinity retinoid binding site was mapped to the cysteine-rich regions in the regulatory domains of Ser/Thr kinases (49). Remarkably, metabolism of vitamin A (retinol) increases in heart after myocardial infarction (89).

In the N-terminal regulatory domain, the 50 amino acid-long, highly homologous stretches containing six conserved cysteine and two conserved histidine residues, tetrahedrally coordinated by two Zn^{2+} ions into a composite zinc finger are targets of redox control (113). When oxidized, the autoinhibitory function of the regulatory domain is compromised and, consequently, cellular PKC activity is stimulated. This topological change is believed to be complemented by phosphorylation, translocation, and Ca^{2+} and phosphatidylserine binding, to lock the catalytic domain into an active form. As a part of crosstalk, PKCδ becomes phosphorylated on Tyr at residues 512 and 523 under the conditions of oxidative stress (67).

Remarkably, both classical and redox activation of PKC trigger the same event, namely zinc release from the regulatory domain. Phorbol esters mimic the action of second messenger diacylglycerol by binding to the same motif within regulatory domain and activating catalytic activity. Neonatal rat cardiomyocytes treated with PMA and with hydrogen peroxide released substantial amounts of Zn^{2+}, which could be detected within minutes of the treatment as visualized by confocal microscopy, using TSQ as indicator (68). Of course, oxidation could trigger zinc release from multiple targets. More intriguing is a similar response to classical PKC activation. PMA-induced zinc increase along with the measurements in the cells overexpressing different PKC isoforms may suggest that PKC itself, as well as PKC-triggered signaling events, are among the sources of free intracellular Zn^{2+} (illustrated in Fig. 3). The proof was obtained experimentally by evaluating *in vitro* the glutathione-*S*-transferase fusion proteins, containing the human PKCδ C1A, δC1B, ζC1, or cRaf C1 peptides using a spectrofluorimetric or the commonly used colorimetric based on 4-(2-pyridylazo-resorcinol) colorimetric quantitative assays. Both 1.3-diolein and PMA triggered the release

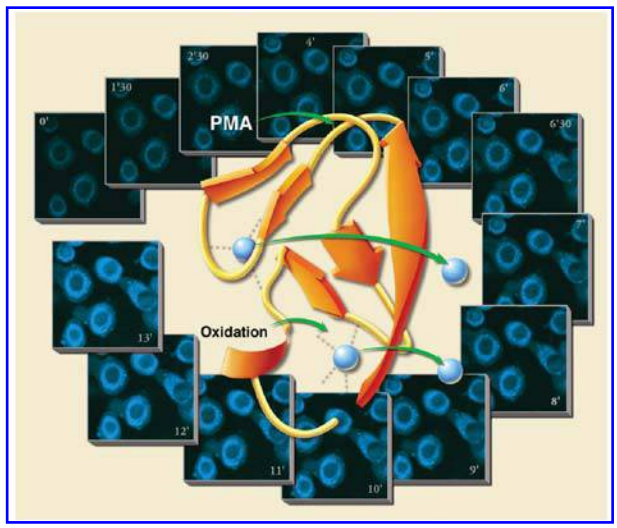

FIG. 3. Zinc release from protein kinase C. Confocal images of insect cells overexpressing PKCα show increase of free Zn^{2+} over time with PMA treatment. Structural properties of PKC zinc finger: the additive effect of oxidants and lipid activators on the release of chelated zinc. (Adapted from Korichneva *et al*. 2002). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

of stoichiometric amounts of zinc, each mole of lipid generating one equivalent of free Zn^{2+} (69). Although the exact number of the binding constant of Zn^{2+} to PKC zinc finger is not available from our measurements, it can be estimated to exceed that of the probes, TSQ, and PAR. Upon oxidation, or PMA binding to the cys domain, the chelation status of Zn^{2+} is changed, allowing Zn^{2+} to partition to the probes. Indeed, in a separate study the electrospray ionization mass spectrometry allowed to determine dissociation constant of zinc from the zinc finger of 0.66 n*M* (100).

Since the cells in culture may change certain phenotypic features, we did confirm our observations of PMA-triggered zinc release on the model of whole Langendorff perfused rat hearts by monitoring tissue sections after ischemia/reperfusion. In addition, our results suggested that heart tissue becomes zinc-depleted after ischemia/reperfusion. More importantly, the capacity to liberate labile zinc was significantly diminished (68).

The concept that zinc movements are important for PKC to gain enzymatic capacity was validated by comparing the Zn^{2+} contents in resting and active PKC, with the expectation that the former should contain more Zn^{2+}. Indeed, zinc content correlated inversely with PKC activity (69). These results are in complete agreement with those of Knapp and Kann (64) who had previously underscored the importance of zinc release during PKC activation by the redox mechanism. Altogether, the data present the evidence defining cysteine-rich domain of the kinase as a redox sensor and a reversible redox zinc switch.

It is unclear how the loss of zinc during PKC activating agrees with the fact that PKC is activated by zinc in many experiments. To solve this controversy, based on the published accounts and our unpublished data, we suggest a possible explanation (Fig. 4). The model advocating the dynamic regulatory role for zinc ions is in agreement with zinc release upon one activation cycle. However, liberated zinc cannot diffuse far away; rather it needs to be in close proximity for the recovery of the kinase. There are three possible scenarios in this instance: free zinc is compartmentalized within the kinase domain, it can bind with lower affinity to another site on the kinase to be available upon reduction of sulfur ligand, or it can repartition to a kinase partner that would provide the ion to the kinase, not to miss the next message. The reversible activation would be a normal physiological response to stress, while irreversible overoxidation would trigger protein degradation, and consequently apoptosis.

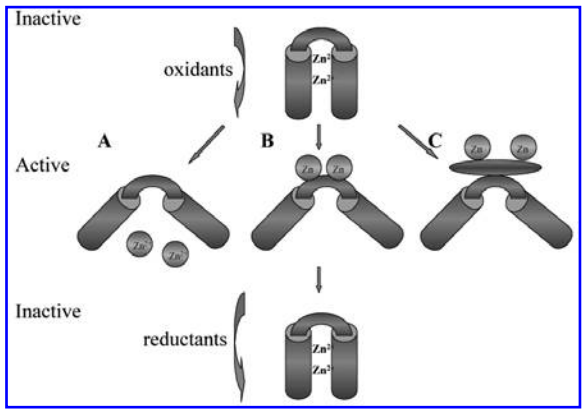

FIG. 4. Hypothetical model of PKC activation by zinc release. Oxidation of cysteines triggers zinc release and unfolds the kinase into an active conformation. The three possible scenarios embrace free zinc compartmentalization within the kinase domain (**A**), zinc binding to the kinase with lower affinity to another site on the kinase (**B**), or participation of a partner to which zinc can repartition during one oxidation cycle (**C**). The proximity of zinc ions to the regulatory domain is required for conformational recovery that allows the kinase to respond to the next message. Irreversible oxidation would trigger protein degradation.

On the whole, zinc coordination centers of the kinases represent a redox zinc switch mechanism that is more complex than described earlier for bacterial chaperone (8). Zinc ions would function as linchpins in that switch to start the clock, initiating precisely the kind of conformational changes that have been postulated by others for the commencement of PKC catalytic activity. These changes involve the removal of the regulatory N-terminus from the catalytic domain, the exposure of hydrophobic surfaces that facilitate translocation to membranes, and the accessibility to substrate and cofactors. The importance of the mechanism of the reversible redox zinc switch is highlighted by the fact that the two signaling pathways of PKC activation converge on the zinc finger. Due to the oxidation of cysteines, or by affecting zinc affinity to histidine via the chain of hydrogen bonds and consequently redox microenvironment of cysteine cluster (in case of PMA), the two different stimuli cause principally the same effect.

It is very probable that other serine/threonine kinases sharing high homology in a composite zinc finger are operated by similar redox zinc switch. Along with different PKC isoforms, they include Raf-1, Rac1 exchange factor Vav (115), chimaerins, Rac-GTPas-activating protein (11), and others. The two pathways, although utilizing messengers of different chemical nature, trigger basically the same event—zinc release from the regulatory domain thus converging on the zinc finger of the kinase. In the equilibrium of oxidants and antioxidants, "Redox Status of Individual Molecules," specifically the status of zinc cysteine cluster may become the most valid biomarker of a stressed tissue and its capacities to respond to stimuli. Possibly the most effective protection from ischemic damage, preconditioning sets up the redox zinc switch on the vital regulatory molecules. Better knowledge of the operation of this switch would help to find the clue to the mechanism of such protection.

CONCLUDING REMARKS

The complexity of the signaling system based on zinc has been partially deconvoluted over the past several years. An important part of the puzzle, one that reveals the major players in zinc homeostasis, is now solved, leaving more questions than answers, particularly relating to the tissue-specific role of these regulators. Here are a few:

(a) Based on the experimental evidence of regulation by ZnT of LTCC, it could be of immense importance to verify the phenomenon in cardiac tissue, where LTCC is the key regulator of conductance. Effects of zinc on

calcium homeostasis are to be studied in detail. In regard to zinc transport, the cross-regulation by different ion transporting systems should be examined.

(b) Since zinc effect on mitochondrial function is tissue specific, zinc targets in heart mitochondria must be characterized.

(c) We still need to understand the phenotype of MT knockout mouse model. It would be required to turn off all of the isoforms to confirm the exceptional role of MT as a zinc storage protein and a zinc donor under critical conditions.

(d) We need to develop a better understanding of zincosomes. How different they are from calcium storage compartments? What are their similarities?

(e) The recognition of temporal and spatial fluctuation of intracellular zinc suggests its potential role in signal transduction. We need to expand the record of the proteins that are regulated by the redox zinc switch.

(f) Finally, we definitively need to proceed with the creation of new faster, more selective, and more sensitive probes for zinc detection.

These and many more questions are within the area of exploration over the next decade. The diversity of zinc functions is fascinating, and it is promising for future biomedical and pharmacological research. The success would be determined by picking the right target and understanding the biology of the target. The new knowledge in the area of zinc homeostasis will improve our understanding of cardiac disorders and suggest new strategies for pharmacological interventions.

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ABBREVIATIONS

CuZnSOD, copper–zinc superoxide dismutase; DAG, diacylglycerol; MRE, metal response element; MT metallothionein; MTF, metal responsive transcription factor; PDE, cyclic nucleotide phosphodiesterases; PKC, protein kinase C; PMA, phorbol-myristate-acetate; PTP, permeability transition pore; PTPs, protein tyrosine phosphatases; SLC, solute-linked carrier; T, thionein; TPEN, *N′*-tetrakis(2-pyridylmethyl)ethylenediamine; TSQ, *N*-(6-methoxy-8-quinolyl)-p-toluensulfonamide.

REFERENCES

1. Andreka P, Nadhazi Z, Muzes G, Szantho G, Vandor L, Konya L, Turner MS, Tulassay Z, and Bishopric NH. Possible therapeutic targets in cardiac myocyte apoptosis. *Curr Pharm Des* 10: 2445–2461, 2004.
2. Apostolova MD, Ivanova IA, and Cherian MG. Metallothionein and apoptosis during differentiation of myoblasts to myotubes: protection against free radical toxicity. *Toxicol Appl Pharmacol* 159: 175–184, 1999.
3. Arnesano F, Banci L, Bertini I, Martinelli M, Furukawa Y, and O'Halloran TV. The unusually stable quaternary structure of human Cu,Zn-superoxide dismutase 1 is controlled by both metal occupancy and disulfide status. *J Biol Chem* 279: 47998–48003, 2004.
4. Atar D, Backx PH, Appel MM, Gao WD, and Marban E. Excitation-transcription coupling mediated by zinc influx through voltage-dependent calcium channels. *J Biol Chem* 270: 2473–2477, 1995.
5. Ayaz M and Turan B. Selenium prevents diabetes-induced alterations in [Zn2+]i and metallothionein level of rat heart via restoration of cell redox cycle. *Am J Physiol Heart Circ Physiol* 290: H1071–H1080, 2006.
6. Azriel–Tamir H, Sharir H, Schwartz B, and Hershfinkel M. Extracellular zinc triggers ERK-dependent activation of Na+/H+ exchange in colonocytes mediated by the zinc-sensing receptor. *J Biol Chem* 279: 51804–51816, 2004.
7. Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL, Guo Y, Bolli R, Cardwell EM, and Ping P. Protein kinase Cepsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. *Circ Res* 92: 873–880, 2003.
8. Barbirz S, Jakob U, and Glocker MO. Mass spectrometry unravels disulfide bond formation as the mechanism that activates a molecular chaperone. *J Biol Chem* 275: 18759–18766, 2000.
9. Bossy–Wetzel E, Talantova MV, Lee WD, Scholzke MN, Harrop A, Mathews E, Gotz T, Han J, Ellisman MH, Perkins GA, and Lipton SA. Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K+ channels. *Neuron* 41: 351–365, 2004.
10. Brown AM, Kristal BS, Effron MS, Shestopalov AI, Ullucci PA, Sheu KF, Blass JP, and Cooper AJ. Zn2+ inhibits alpha-ketoglutarate-stimulated mitochondrial respiration and the isolated alpha-ketoglutarate dehydrogenase complex. *J Biol Chem* 275: 13441–13447, 2000.
11. Caloca MJ, Garcia–Bermejo ML, Blumberg PM, Lewin NE, Kremmer E, Mischak H, Wang S, Nacro K, Bienfait B, Marquez VE, and Kazanietz MG. beta2-chimaerin is a novel target for diacylglycerol: binding properties and changes in subcellular localization mediated by ligand binding to its C1 domain. *Proc Natl Acad Sci USA* 96: 11854–11859, 1999.
12. Chen CH, Gray MO, and Mochly–Rosen D. Cardioprotection from ischemia by a brief exposure to physiological levels of ethanol: role of epsilon protein kinase C. *Proc Natl Acad Sci USA* 96: 12784–12789, 1999.
13. Cheung PY, Sawicki G, WoZniak M, Wang W, Radomski MW, and Schulz R. Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart. *Circulation* 101: 1833–1839, 2000.
14. Chou SS, Clegg MS, Momma TY, Niles BJ, Duffy JY, Daston GP, and Keen CL. Alterations in protein kinase C activity and processing during zinc-deficiency-induced cell death. *Biochem J* 383: 63–71, 2004.
15. Clegg MS, Hanna LA, Niles BJ, Momma TY, and Keen CL. Zinc deficiency-induced cell death. *IUBMB Life* 57: 661–669, 2005.

16. Coudray C, Charlon V, de Leiris J, and Favier A. Effect of zinc deficiency on lipid peroxidation status and infarct size in rat hearts. *Int J Cardiol* 41: 109–113, 1993.
17. Cragg RA, Christie GR, Phillips SR, Russi RM, Kury S, Mathers JC, Taylor PM, and Ford D. A novel zinc-regulated human zinc transporter, hZTL1, is localized to the enterocyte apical membrane. *J Biol Chem* 77: 22789–22797, 2002.
18. Cross HR, Murphy E, Bolli R, Ping P, and Steenbergen C. Expression of activated PKC epsilon (PKC epsilon) protects the ischemic heart, without attenuating ischemic H(+) production. *J Mol Cell Cardiol* 34: 361–367, 2002.
19. Cruzado MC, Risler NR, Miatello RM, Yao G, Schiffrin EL, and Touyz RM. Vascular smooth muscle cell NAD(P)H oxidase activity during the development of hypertension: effect of angiotensin II and role of insulin like growth factor-1 receptor transactivation. *Am J Hypertens* 18: 81–87, 2005.
20. Das DK, Maulik N, and Engelman RM. Redox regulation of angiotensin II signaling in the heart. *J Cell Mol Med* 8: 144–152, 2004.
21. Devirgiliis C, Murgia C, Danscher G, and Perozzi G. Exchangeable zinc ions transiently accumulate in a vesicular compartment in the yeast *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 323: 58–64, 2004.
22. Disatnik MH, Buraggi G, and Mochly–Rosen D. Localization of protein kinase C isozymes in cardiac myocytes. *Exp Cell Res* 210: 287–297, 1994.
23. Duffy JY, Overmann GJ, Keen CL, Clegg MS, and Daston GP. Cardiac abnormalities induced by zinc deficiency are associated with alterations in the expression of genes regulated by the zinc-finger transcription factor GATA-4. *Birth Defects Res B Dev Reprod Toxicol* 71: 102–109, 2004.
24. Eide DJ. The SLC39 family of metal ion transporters. *Pflugers Arch* 447: 796–800, 2004.
25. el-Tanani MK and Green CD. Insulin/IGF-1 modulation of the expression of two estrogen-induced genes in MCF-7 cells. *Mol Cell Endocrinol* 121: 29–35, 1996.
26. Ekin S, Mert N, Gunduz H, and Meral I. Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. *Biol Trace Elem Res.* 94: 193–201, 2003.
27. Fabisiak JP, Borisenko GG, Liu SX, Tyurin VA, Pitt BR, and Kagan VE. Redox sensor function of metallothioneins. *Methods Enzymol* 353: 268–281, 2002.
28. Failla ML and Cousins RJ. Zinc uptake by isolated rat liver parenchymal cells. *Biochim Biophys Acta* 538: 435–444, 1978.
29. Feener EP, Northrup JM, Aiello LP, and King GL. Angiotensin II induces plasminogen activator inhibitor-1 and -2 expression in vascular endothelial and smooth muscle cells. *J Clin Invest* 95: 1353–1362, 1995.
30. Feng W, Cai J, Pierce WM, Franklin RB, Maret W, Benz FW, and Kang YJ. Metallothionein transfers zinc to mitochondrial aconitase through a direct interaction in mouse hearts. *Biochem Biophys Res Commun* 332: 853–858, 2005.
31. Feng W, Benz FW, Cai J, Pierce WM, and Kang YJ. Metallothionein disulfides are present in metallothionein-overexpressing transgenic mouse heart and increase under conditions of oxidative stress. *J Biol Chem* 281: 681–687, 2006.
32. Fernandez-Patron C, Radomski MW, and Davidge ST. Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor. *Circ Res* 85: 906–911, 1999.
33. FernandezPatron C, Stewart KG, Zhang Y, Koivunen E, Radomski MW, and Davidge ST. Vascular matrix metalloproteinase-2-dependent cleavage of calcitonin gene-related peptide promotes vasoconstriction. *Circ Res* 87: 670–676, 2000.
34. Forbes IJ, Zalewski PD, Giannakis C, Petkoff HS, and Cowled PA. Interaction between protein kinase C and regulatory ligand is enhanced by a chelatable pool of cellular zinc. *Biochim Biophys Acta* 1053: 113–117, 1990.
35. Forbes IJ, Zalewski PD, Giannakis C, and Betts WH. Zinc induces specific association of PKC with membrane cytoskeleton. *Biochem Int* 22: 741–748, 1990.
36. Forbes IJ, Zalewski PD, and Giannakis C. Role for zinc in a cellular response mediated by protein kinase C in human B lymphocytes. *Exp Cell Res* 195: 224–229, 1991.
37. Francis SH, Colbran JL, McAllister–Lucas LM, and Corbin JD. Zinc interactions and conserved motifs of the cGMP-binding cGMP-specific phosphodiesterase suggest that it is a zinc hydrolase. *J Biol Chem* 269: 22477–22480, 1994.
38. Gao CQ, Sawicki G, Suarez–Pinzon WL, Csont T, Wozniak M, Ferdinandy P, and Schulz R. Matrix metalloproteinase-2 mediates cytokine-induced myocardial contractile dysfunction. *Cardiovasc Res* 57: 426–433, 2003.
39. Goldstein BJ, Kalyankar M, and Wu X. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 54: 311–321, 2005.
40. Gopalakrishna R and Anderson WB. Ca2+- and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. *Proc Natl Acad Sci USA* 86: 6758–6762, 1989.
41. Gopalakrishna R and Jaken S. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 28: 1349–1361, 2000.
42. Gore A, Moran A, Hershfinkel M, and Sekler I. Inhibitory mechanism of store-operated Ca2+ channels by zinc. *J Biol Chem* 279: 11106–11111, 2004.
43. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, and Lipton SA. *S*-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* 297: 1186–1190, 2002.
44. Haase H and Beyersmann D. Intracellular zinc distribution and transport in C6 rat glioma cells. *Biochem Biophys Res Commun* 296: 923–928, 2002.
45. Haase H and Maret W. Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. *Exp Cell Res* 291: 289–298, 2003.
46. Hainaut P and Mann K. Zinc binding and redox control of p53 structure and function. *Antioxid Redox Signal* 3: 611–623, 2003.
47. Harris FM, Brecht WJ, Xu Q, Mahley RW, and Huang Y. Increased tau phosphorylation in apolipoprotein E4 trans-

genic mice is associated with activation of extracellular signal-regulated kinase: modulation by zinc. *J Biol Chem* 279: 44795–44801, 2004.
48. Hershfinkel M, Moran A, Grossman N, and Sekler I. A zinc-sensing receptor triggers the release of intracellular Ca2+ and regulates ion transport. *Proc Natl Acad Sci USA* 98: 11749–11754, 2001.
49. Imam A, Hoyos B, Swenson C, Levi E, Chua R, Viriya E, and Hammerling U. Retinoids as ligands and coactivators of protein kinase C alpha. *FASEB J* 15: 28–30, 2001.
50. Inoue K, Matsuda K, Itoh M, Kawaguchi H, Tomoike H, Aoyagi T, Nagai R, Hori M, Nakamura Y, and Tanaka T. Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene, ZnT-5. *Hum Mol Genet* 11: 1775–1784, 2002.
51. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 409: 860–921, 2001.
52. Iwai–Kanai E and Hasegawa K. Intracellular signaling pathways for norepinephrine- and endothelin-1-mediated regulation of myocardial cell apoptosis. *Mol Cell Biochem* 259: 163–168, 2004.
53. Jacob C, Maret W, and Vallee BL. Control of zinc transfer between thionein, metallothionein, and zinc proteins. *Proc Natl Acad Sci USA* 95: 3489–3494, 1998.
54. Jakob U, Eser M, and Bardwell JC. Redox switch of hsp33 has a novel zinc-binding motif. *J Biol Chem* 275: 38302–38310, 2000.
55. Jiang H, Daniels PJ, and Andrews GK. Putative zinc-sensing zinc fingers of metal-response element-binding transcription factor-1 stabilize a metal-dependent chromatin complex on the endogenous metallothionein-I promoter. *J Biol Chem* 278: 30394–303402, 2003.
56. Jiang LJ, Vasak M, Vallee BL, and Maret W. Zinc transfer potentials of the alpha- and beta-clusters of metallothionein are affected by domain interactions in the whole molecule. *Proc Natl Acad Sci USA* 97: 2503–2508, 2000.
57. Kakita T, Hasegawa K, Iwai–Kanai E, Adachi S, Morimoto T, Wada H, Kawamura T, Yanazume T, and Sasayama S. Calcineurin pathway is required for endothelin-1-mediated protection against oxidant stress-induced apoptosis in cardiac myocytes. *Circ Res* 88: 1239–1246, 2001.
58. Kambe T, Narita H, Yamaguchi–Iwai Y, Hirose J, Amano T, Sugiura N, Sasaki R, Mori K, Iwanaga T, and Nagao M. Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic beta cells. *J Biol Chem* 277: 19049–19055, 2002.
59. Kang YJ. The antioxidant function of metallothionein in the heart. *Proc Soc Exp Biol Med* 222: 263–273, 1999.
60. Kang YJ, Li Y, Sun X, and Sun X. Antiapoptotic effect and inhibition of ischemia/reperfusion-induced myocardial injury in metallothionein-overexpressing transgenic mice. *Am J Pathol* 163: 1579–1586, 2003.
61. Kawamura S, Yoshida K, Miura T, Mizukami Y, and Matsuzak M. Ischemic preconditioning translocates PKC-delta and -epsilon, which mediate functional protection in isolated rat heart. *Am J Physiol* 275: H2266–2271, 1998.
62. Kerchner GA, Canzoniero LM, Yu SP, Ling C, and Choi DW. Zn2+ current is mediated by voltage-gated Ca2+ channels and enhanced by extracellular acidity in mouse cortical neurones. *J Physiol* 528: 39–52, 2000.
63. Klein C, Heyduk T, and Sunahara RK. Zinc inhibition of adenylyl cyclase correlates with conformational changes in the enzyme. *Cell Signal* 16: 1177–1185, 2004.
64. Knapp LT and Klann E. Superoxide-induced stimulation of protein kinase C via thiol modification and modulation of zinc content. *J Biol Chem* 275: 24136–24145, 2000.
65. Koizumi S, Suzuki K, Ogra Y, Gong P, and Otuska F. Roles of zinc fingers and other regions of the transcription factor human MTF-1 in zinc-regulated DNA binding. *J Cell Physiol* 185: 464–472, 2000.
66. Kondoh M, Tasaki E, Takiguchi M, Higashimoto M, Watanabe Y, and Sato M. Activation of caspase-3 in HL-60 cells treated with pyrithione and zinc. *Biol Pharm Bull* 28: 757–759, 2005.
67. Konishi H, Tanaka M, Takemura Y, Matsuzaki H, Ono Y, Kikkawa U, and Nishizuka Y. Activation of protein kinase C by tyrosine phosphorylation in response to H2O2. *Proc Natl Acad Sci USA* 94: 11233–11237, 1997.
68. Korichneva I. Redox regulation of cardiac protein kinase C. *Exp Clin Cardiol* 10: 256–261, 2005.
69. Korichneva I, Hoyos B, Chua R, Levi E, and Hammerling U. Zinc release from protein kinase C as the common event during activation by lipid second messenger or reactive oxygen. *J Biol Chem* 277: 44327–44331, 2002.
70. Kown MH, Van der Steenhoven T, Blankenberg FG, Hoyt G, Berry GJ, Tait JF, Strauss HW, and Robbins RC. Zinc-mediated reduction of apoptosis in cardiac allografts. *Circulation* 102: III228–232, 2000.
71. Kresse W, Sekler I, Hoffmann A, Peters O, Nolte C, Moran A, and Kettenmann H. Zinc ions are endogenous modulators of neurotransmitter-stimulated capacitative Ca2+ entry in both cultured and *in situ* mouse astrocytes. *Eur J Neurosci* 21: 1626–1634, 2005.
72. Kroncke KD. Zinc finger proteins as molecular targets for nitric oxide-mediated gene regulation. *Antioxid Redox Signal* 3: 565–575, 2001.
73. Kuznetsova SS, Azarkina NV, Vygodina TV, Siletsky SA, and Konstantinov AA. Zinc ions as cytochrome C oxidase inhibitors: two sites of action. *Biochemistry (Mosc)* 70: 128–136, 2005.
74. Langmade SJ, Ravindra R, Daniels PJ, and Andrews GK. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. *J Biol Chem* 275: 34803–34809, 2000.
75. Liu JL, Rigolet P, Dou SX, Wang PY, and Xi XG. The zinc finger motif of Escherichia coli RecQ is implicated in both DNA binding and protein folding. *J Biol Chem* 279: 42794–427802, 2004.
76. Liuzzi JP, Blanchard RK, and Cousins RJ. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. *J Nutr* 131: 46–52, 2001.
77. Liuzzi JP and Cousins RJ. Mammalian zinc transporters. *Annu Rev Nutr* 24: 151–172, 2004.
78. Maret W. Zinc and sulfur: a critical biological partnership. *Biochemistry* 43: 3301–3309, 2004.
79. Maret W and Vallee BL. Thiolate ligands in metallothionein confer redox activity on zinc clusters. *Proc Natl Acad Sci USA* 95: 3478–3482, 1998.

80. Matsusaka H, Ide T, Matsushima S, Ikeuchi M, Kubota T, Sunagawa K, Kinugawa S, and Tsutsui H. Targeted deletion of p53 prevents cardiac rupture after myocardial infarction in mice. *Cardiovasc Res* 113: 1779–1786, 2006.
81. Michalczyk AA, Allen J, Blomeley RC, and Ackland ML. Constitutive expression of hZnT4 zinc transporter in human breast epithelial cells. *Biochem J* 364: 105–113, 2002.
82. Mooradian AD, Morley JE, and Scarpace PJ. The role of zinc status in altered cardiac adenylate cyclase activity in diabetic rats. *Acta Endocrinol (Copenh)* 119: 174–180, 1988.
83. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem* 378: 151–160, 1997.
84. Naruse K and King G. Protein kinase C and myocardial biology and function. *Circ Res* 86: 1104–1106, 2000.
85. Ohana E, Segal D, Palty R, Ton–That D, Moran A, Sensi SL, Weiss JH, Hershfinkel M, and Sekler I. A sodium zinc exchange mechanism is mediating extrusion of zinc in mammalian cells. *J Biol Chem* 279: 4278–4284, 2004.
86. Oteiza PI and Mackenzie GG. Zinc, oxidant-triggered cell signaling, and human health. *Mol Aspects Med* 26: 245–255, 2005.
87. Otvos JD, Liu X, Li H, Shen G, and Basti M. Dynamic aspects of metallothionein structure. In: *Metallothionein III,* Suzuki KT, Imura N, and Kimura M (eds). Basel, Switzerland: Birkhäuser, 1993, pp.57–74.
88. Pacher P, Schulz R, Liaudet L, and Szabo C. Nitrosative stress and pharmacological modulation of heart failure. *Trends Pharmacol Sci* 26: 302–310, 2005.
89. Palace VP, Hill MF, Khaper N, and Singal PK. Metabolism of vitamin A in the heart increases after a myocardial infarction. *Free Radic Biol Med* 26: 1501–1507, 1999.
90. Palmiter RD and Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J* 14: 639–649, 1995.
91. Palmiter RD and Huang L. Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. *Pflugers Arch* 447: 744–751, 2004.
92. Pines A, Bivi N, Romanello M, Damante G, Kelley MR, Adamson ED, D'Andrea P, Quadrifoglio F, Moro L, and Tell G. Cross-regulation between Egr-1 and APE/Ref-1 during early response to oxidative stress in the human osteoblastic HOBIT cell line: evidence for an autoregulatory loop. *Free Radic Res* 39: 269–281, 2005.
93. Powell SR, Aiuto L, Hall D, and Tortolani AJ. Zinc supplementation enhances the effectiveness of St. Thomas' Hospital No. 2 cardioplegic solution in an *in vitro* model of hypothermic cardiac arrest. *J Thorac Cardiovasc Surg* 110: 1642–1648, 1995.
94. Regula KM and Kirshenbaum LA. Apoptosis of ventricular myocytes: a means to an end. *J Mol Cell Cardiol* 38: 3–13, 2005.
95. Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. *Am J Clin Nutr* 61(3 Suppl): 621S–624S, 1995.
96. Sawicki G, Leon H, Sawicka J, Sariahmetoglu M, Schulze CJ, Scott PG, Szczesna–Cordary D, and Schulz R. Degradation of myosin light chain in isolated rat hearts subjected to ischemia-reperfusion injury: a new intracellular target for matrix metalloproteinase-2. *Circulation* 112: 544–552, 2005.
97. Segal D, Ohana E, Besser L, Hershfinkel M, Moran A, and Sekler I. A role for ZnT-1 in regulating cellular cation influx. *Biochem Biophys Res Commun* 323: 1145–1150, 2004.
98. Sensi SL and Jeng JM. Rethinking the excitotoxic ionic milieu: the emerging role of Zn(2+) in ischemic neuronal injury. *Curr Mol Med* 4: 87–111, 2004.
99. Sensi SL, Ton–That D, Sullivan PG, Jonas EA, Gee KR, Kaczmarek LK, and Weiss JH. Modulation of mitochondrial function by endogenous Zn2+ pools. *Proc Natl Acad Sci USA* 100: 6157–6162, 2003.
100. Shindo M, Irie K, Fukuda H, and Ohigashi H. Analysis of the non-covalent between metal ions and the cysteine-rich domain of protein kinase C eta by electrospray ionization mass spectrometry. *Bioorg Med Chem* 23: 5075–5072, 2003.
101. Silke J and Vaux DL. Two kinds of BIR-containing protein–inhibitors of apoptosis, or required for mitosis. *J Cell Sci* 14: 1821–1827, 2001.
102. Steinebach OM and Wolterbeek BT. Metallothionein biodegradation in rat hepatoma cells: a compartmental analysis aided 35S-radiotracer study. *Biochim Biophys Acta* 1116: 155–165, 1992.
103. Sun Y, Tan M, Duan H, and Swaroop M. SAG/ROC/Rbx/Hrt, a zinc RING finger gene family: molecular cloning, biochemical properties, and biological functions. *Antioxid Redox Signal* 3: 635–650, 2001.
104. Taylor KM and Nicholson RI. The LZT proteins; the LIV-1 subfamily of zinc transporters. *Biochim Biophys Acta* 1611: 16–30, 2003.
105. Tenhunen O, Sarman B, Kerkela R, Szokodi I, Papp L, Toth M, and Ruskoaho H. Mitogen-activated protein kinases p38 and ERK 1/2 mediate the wall stress-induced activation of GATA-4 binding in adult heart. *J Biol Chem* 279: 24852–24860, 2004.
106. Truong–Tran AQ, Grosser D, Ruffin RE, Murgia C, and Zalewski PD. Apoptosis in the normal and inflamed airway epithelium: role of zinc in epithelial protection and procaspase-3 regulation. *Biochem Pharmacol* 66: 1459–1468, 2003.
107. Vallee BL and Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev* 73: 79–118, 1993.
108. Wang W, Sawicki G, and Schulz R. Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. *Cardiovasc Res* 53: 165–174, 2002.
109. Wang W, Schulze CJ, Suarez–Pinzon WL, Dyck JR, Sawicki G, and Schulz R. Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. *Circulation* 106: 1543–1549, 2002.
110. Wang J, Song Y, Elsherif L, Song Z, Zhou G, Prabhu SD, Saari JT, and Cai L. Cardiac metallothionein induction plays the major role in the prevention of diabetic cardiomyopathy by zinc supplementation. *Circulation* 113: 544–554, 2006.
111. Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, and King GL. Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms,

and endothelial cell growth. *J Clin Invest* 98: 2018–2026, 1996.
112. Yang Y, Maret W, and Vallee BL. Differential fluorescence labeling of cysteinyl clusters uncovers high tissue levels of thionein. *Proc Natl Acad Sci USA* 98: 5556–5559, 2001.
113. Zhang G, Kazanietz MG, Blumberg PM, and Hurley JH. Crystal structure of the cys2 activator-binding domain of protein kinase C delta in complex with phorbol ester. *Cell* 81: 917–924, 1995.
114. Zou MH, Shi C, and Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest* 109: 817–826, 2002.
115. Zugaza JL, Lopez–Lago MA, Caloca MJ, Dosil M, Movilla N, and Bustelo XR. Structural determinants for the biological activity of Vav proteins. *J Biol Chem* 277: 45377–45392, 2002.

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2. Claudia Penna , Maria-Giulia Perrelli , Pasquale Pagliaro . Mitochondrial Pathways, Permeability Transition Pore, and Redox Signaling in Cardioprotection: Therapeutic Implications. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
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5. Pasquale Pagliaro , Francesca Moro , Francesca Tullio , Maria-Giulia Perrelli , Claudia Penna . 2011. Cardioprotective Pathways During Reperfusion: Focus on Redox Signaling and Other Modalities of Cell Signaling. *Antioxidants & Redox Signaling* **14**:5, 833-850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Peter J. Little, Runa Bhattacharya, Abel E. Moreyra, Irina L. Korichneva. 2010. Zinc and cardiovascular disease. *Nutrition* **26**:11-12, 1050-1057. [CrossRef]
7. Rachel McIntosh, SungRyul Lee, Andrew J. Ghio, Jinkun Xi, Min Zhu, Xiangjun Shen, Guillaume Chanoit, David A. Zvara, Zhelong Xu. 2010. The critical role of intracellular zinc in adenosine A2 receptor activation induced cardioprotection against reperfusion injury. *Journal of Molecular and Cellular Cardiology* **49**:1, 41-47. [CrossRef]
8. Guy Vassort, Belma Turan. 2010. Protective Role of Antioxidants in Diabetes-Induced Cardiac Dysfunction. *Cardiovascular Toxicology* **10**:2, 73-86. [CrossRef]
9. Adrian C. Nicolescu, Andrew Holt, Arulmozhi D. Kandasamy, Pal Pacher, Richard Schulz. 2009. Inhibition of matrix metalloproteinase-2 by PARP inhibitors. *Biochemical and Biophysical Research Communications* **387**:4, 646-650. [CrossRef]
10. Claudia Penna, Daniele Mancardi, Raffaella Rastaldo, Pasquale Pagliaro. 2009. Cardioprotection: A radical view. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1787**:7, 781-793. [CrossRef]
11. V. Ansteinsson, M. Refsnes, T. Skomedal, J. B. Osnes, I. Schiander, M. Låg. 2009. Zinc- and Copper-Induced Interleukin-6 Release in Primary Cell Cultures From Rat Heart. *Cardiovascular Toxicology* **9**:2, 86-94. [CrossRef]
12. Jayna Chan, Zuyun Huang, Ian Watt, Peter Kille, Martin Stillman. 2008. Metallobiological Necklaces: Mass Spectrometric and Molecular Modeling Study of Metallation in Concatenated Domains of Metallothionein. *Chemistry - A European Journal* **14**:25, 7579-7593. [CrossRef]
13. Junichi Sadoshima . 2006. Redox Regulation of Growth and Death in Cardiac Myocytes. *Antioxidants & Redox Signaling* **8**:9-10, 1621-1624. [Citation] [Full Text PDF] [Full Text PDF with Links]