Review

Calcium and oxidative stress: from cell signaling to cell death

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Abstract

Reactive oxygen and nitrogen species can be used as a messengers in normal cell functions. However, at oxidative stress levels they can disrupt normal physiological pathways and cause cell death. Such a switch is largely mediated through Ca^{2+} signaling. Oxidative stress causes Ca^{2+} influx into the cytoplasm from the extracellular environment and from the endoplasmic reticulum or sarcoplasmic reticulum (ER/SR) through the cell membrane and the ER/SR channels, respectively. Rising Ca^{2+} concentration in the cytoplasm causes Ca^{2+} influx into mitochondria and nuclei. In mitochondria Ca^{2+} accelerates and disrupts normal metabolism leading to cell death. In nuclei Ca^{2+} modulates gene transcription and nucleases that control cell apoptosis. Both in nuclei and cytoplasm Ca^{2+} can regulate phosphorylation/dephosphorylation of proteins and can modulate signal transduction pathways as a result. Since oxidative stress is associated with many diseases and the aging process, understanding how oxidants alter Ca^{2+} signaling can help to understand process of aging and disease, and may lead to new strategies for their prevention. © 2002 Elsevier Science Ltd. All rights reserved.

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Contents

1. Introduction

The biological functions of Ca^{2+} are incredibly versatile; they control multiple processes of birth, life and death. Calcium signals are sent through channels which differ in their cellular location, and in both the amplitude and duration of the response (see Berridge et al., 1998 for review). Location, duration, and amplitude form a complex code that can control vital physiological processes. Any disturbance in these signals would change the "Ca^{2+} code" and modify multiple life processes. Such disturbances can arise from a variety of sources, one of which is oxidative stress. Oxidative stress is characterized as a shift of the cellular redox status to a more oxidized state. Such a shift can be due to exposure of cells to environmental oxidants, or toxins, such as heavy metals that produce reactive oxygen species, ionizing and UV irradiation, heat shock, infections, and others. It also can be due to the endogenous production of reactive oxygen or nitrogen species under pathological conditions such as disease, or during aging. Oxidants can interact with both cellular metabolites and structural elements and modify their properties.

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Such reactions can change the "Ca^{2+} code" and modify essential pathways. In this review we do not intend to describe the basics of calcium signaling, or oxidative stress mechanisms, in detail. These are both well described in the literature, and interested readers are directed to reviews on calcium signaling (Berridge et al., 1998; Santella and Carafoli, 1997; Marin et al., 1999) and reviews on oxidative stress (Davies, 1999, 2000; Pacifici and Davies, 1991; Lander, 1997). Here we would like to discuss how oxidative stress can affect and modify calcium signaling, and explore how oxidants may switch the "Ca^{2+} code" from one that regulates normal cell functions to a signal for cell death.

2. Cytoplasm

Ca^{2+} concentration in the cytoplasm is primarily regulated by Ca^{2+} transport into and out of the endoplasmic reticulum or sarcoplasmic reticulum (ER/SR), in which calcium can be stored, as well as by Ca^{2+} transport through the plasmolemma between cells and their environments (see Berridge et al., 1998; Marin et al., 1999 for review). Calcium can enter the cell cytoplasm through various Ca^{2+} channels, and can be extruded by Ca^{2+} pumps. It also can be transported out of cells through Na^+/Ca^{2+} exchangers, however, under some stress conditions, Ca^{2+} transport can be inhibited or even reversed (see Kaneko et al., 1994). For example, in cardiac myocytes exposed to ischemia/reperfusion, which produces a burst of oxygen radicals, the Na^+/Ca^{2+}

exchanger can operate in the reverse mode causing Ca^{2+} influx into the cytoplasm (Clague and Langer, 1994).

Calcium transport between the ER/SR and the cytoplasm is also regulated by channels, pumps, and Na^+/Ca^{2+} exchangers. The ER/SR store is replenished by Ca^{2+} ATPase pumps and emptied primarily by ligand-operated channels. These channels are sensitive to inositol 1,4,5-triphosphate ($InsP_3$), sensitive to Ca^{2+} itself, to ryanodine, and perhaps to cyclic ADP ribose. Calcium pumps can be inhibited by various pharmacologic and toxic agents, and physiologically they can be regulated by phosphorylation: they are activated when phosphorylated (Matter et al., 1993). Excitable cells such as neurons, also posses voltage-gated Ca^{2+} channels.

It is generally reported that oxidants cause a rapid increase in Ca^{2+} concentration in the cytoplasm of diverse cell types (Roveri et al., 1992; Rooney et al., 1991; Renard et al., 1992; Doan et al., 1994; Joseph et al., 1997; Wang and Joseph, 2000). It seems that this increase can be due to both Ca^{2+} release from internal cellular stores such as the ER/SR, and to Ca^{2+} import from extracellular spaces through the plasmolemma (see Fig. 1), depending on both the concentration and the type of oxidant involved. For example, it has been observed that mild oxidative stress caused by moderate hydrogen peroxide levels transiently increases cytoplasmic Ca^{2+} levels even in Ca^{2+} free medium, but fails to do so if Ca^{2+} is first depleted from the ER/SR (Roveri et al., 1992). Higher concentrations of hydrogen peroxide, however, caused a sustained elevation of cytosolic Ca^{2+} which was not observed in Ca^{2+} free medium, suggesting that
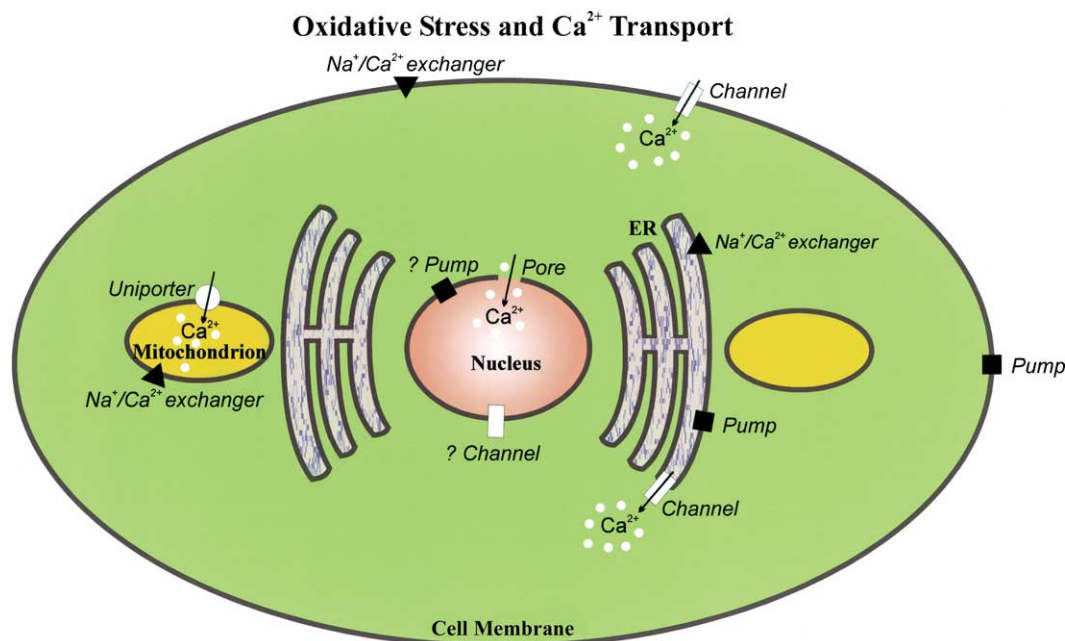

Fig. 1. Oxidative stress and Ca^{2+} transport. Oxidants cause Ca^{2+} influx into the cytoplasm from the extracellular environment and from the ER/SR stores through $InsP_3$-gated channels. At the same time they inhibit Ca^{2+} transport out of the cytoplasm through ATPase pumps. Na^+/Ca^{2+} exchangers can also be inhibited or even reversed, causing additional Ca^{2+} accumulation in the cytoplasm. Rising Ca^{2+} concentration in the cytoplasm leads to Ca^{2+} transport into the mitochondria through uniporters and into the nucleus through pores. The immediate effect of oxidative stress on nuclear Ca^{2+} channels and pumps has not been studied.

severe oxidative stress causes Ca^{2+} uptake by cells from extracellular spaces (Roveri et al., 1992).

Oxidants can directly attack membranes forming lipid peroxides which can activate phospholipase A_2 (PLA_2). The products of PLA_2 action (arachinoic acid and hydroxyl fatty acids) are known as a mediators of such important physiological processes as immune responses and inflamation. It has been demonstrated that lipid peroxides alter Ca^{2+} homeostasis which in turn can modulate PLA_2 activity in endothelial cells (Elliott et al., 1992). This process has been shown to involve protein kinase C (PKC) and $InsP_3$ which we will discuss below. In addition, elevated Ca^{2+} levels in the cytoplasm can activate other enzymes that produce free radicals, such as nitric oxide synthase (Lynch and Dawson, 1994). Thus, by elevating cytoplasmic Ca^{2+} levels, oxidants can also indirectly cause more oxidant production and further escalate Ca^{2+} levels.

2.1. Inositol 1,4,5-triphospate (InsP₃)-operated channels

$InsP_3$ acts on receptors in the ER/SR and causes the release of Ca^{2+} stores. It is generated by the action of phospholipase C on phosphatidylinositol 4,5-biphosphate at the plasma membrane, in response to neurotransmitters or hormones. Oxidants, however, also can regulate production of $InsP_3$ and Ca^{2+} release from the ER/SR as a consequence. For example, hydrogen peroxide can cause release of Ca^{2+} from internal stores in endothelial cells (Doan et al., 1994). Release of Ca^{2+} from the stores by hydrogen peroxide was shown to decline after thapsigargin addition, which supports the contention that hydrogen peroxide accesses $InsP_3$-regulated stores. Similarly, while $InsP_3$ generation was stimulated using $InsP_3$-agonists like bradikinin, oxidants were demonstrated simultaneously to modulate both its levels and calcium signaling. For example, production of $InsP_3$ in response to bradykinin can be significantly enhanced by superoxide anions generated by xanthine oxidase/hypoxanthine (XO/HX) in endothelial cells (Graier et al., 1998). Increased $InsP_3$ production was proposed to cause the increased intracellular Ca^{2+} release and Ca^{2+} entry from extracellular environment. This effect could be prevented by superoxide dismutase, which converts superoxide to hydrogen peroxide.

2.2. Ca²⁺-transporting ATPases

Sarco-endoplasmic reticulum Ca^{2+}-transporting ATPases (SERCA) are ATP-dependent Ca^{2+} pumps that transport Ca^{2+} against its concentration gradient into the lumen of the ER/SR. Thus SERCA antagonize the function of $InsP_3$ by reducing free cytoplasmic calcium levels and accumulating Ca^{2+} in the ER/SR, from where it can later be released through $InsP_3$-operated channels (see Marin et al., 1999 for review). Oxidants such as superoxide (Grover and Samson, 1988) and hydrogen peroxide (Grover et al., 1992) can effectively inhibit Ca^{2+} transport by SERCA pumps in smooth muscle cells. It seems that SERCA can be inhibited both by oxidation of its sylfhydryl groups and by direct attack of oxidants on the ATP binding site (Scherer and Deamer, 1986; Suzuki and Ford, 1991; Xu et al., 1997; Castilho et al., 1996).

Plasma membrane ATPases are also inhibited by oxidants. For example, exposure of red blood cell membranes to ferrous sulfate, which generates hydrogen peroxide and hydroxyl radicals, results in both a concentration- and time-dependent inhibition of the Ca^{2+} pump ATPases (Rohn et al., 1993). This inhibition can be prevented by certain free-radical scavengers including butylated hydroxytoluene and U74006F.

3. Mitochondria

Calcium is transported into mitochondria primarily through uniporters, which might act like channels, opening once local cytoplasmic Ca^{2+} concentrations rise (Rizzuto et al., 2000; Gunter et al., 1998). The re-equilibration Ca^{2+} from mitohondria is largely achieved by mitohondrial Na^+/Ca^{2+} exchangers, which are distinct from those found in the plasmolemma. Under some pathological conditions, for example, hypoxia, these exchangers can be reversed, which would cause calcium transport into mitohondria instead of its release from mitohondria (Griffiths et al., 1998).

Calcium concentrations in mitohondria are dependent on those in the cytoplasm which, in turn depend on Ca^{2+} flux through channels of the ER/SR and the plasma membrane. Mitochondria seem to form a largely interconnected "tubular" network that is in close contact with the ER/SR (Rizzuto et al., 1998). Upon opening of the $InsP_3$-gated channels in the ER/SR, the mitochondrial surface, which is situated in closed proximity to the ER/SR, is exposed to higher concentration of Ca^{2+} than is a bulk cytoplasm, suggesting that mitochondrial Ca^{2+} homeostasis depends on close contact with the ER/SR (see Fig. 2). When the Ca^{2+} concentrations in the cytoplasm rise they will also rise in mitochondria causing activation of mitochondrial metabolism. It seems, however, that under oxidative stress circumstances, mitochondrial Ca^{2+} accumulation can switch from a physiologically beneficial process to a cell death signal. Moreover, high levels of Ca^{2+} in the cytoplasm are not necessary toxic if Ca^{2+} uptake by mitochondria is inhibited. For example, it is well known that ischemic/reperfusion damage to neurons in the central nervous system involves accumulation of the excitatory neurotransmitter, glutamate. Glutamate acts at NMDA receptors in neuronal cells to increase the cytoplasmic Ca^{2+} concentration which in extreme cases, can cause cell death. Concurrent inhibition of Ca^{2+} uptake by mitochondria, however, can significantly reduce cell death (Stout et al., 1998). This protection occurs in spite of the fact that inhibition of mitochondrial Ca^{2+} uptake substantially increases Ca^{2+} concentration in the cytoplasm (Stout et al., 1998), indicating that mitochondria are important targets for switching normal Ca^{2+}

Calcineurin Pathways and Oxidative Stress.

NF-AT 1,4 ⟶ T cell activation

NF-AT3 ⟨ Cardiac muscle development

InsP$_3$-R1 ⟶ Ca^{2+} influx (neuronal cells)

TCN1p ⟶ Vacuolar and secretory Ca^{2+} pumps ⟶ Ca^{2+} infux (yeast)

MEF2 ⟶ Skeletal muscle growth

Oxidative stress ⟶ Ca^{2+} ⟶ CaM ⟶ Calcineurin ⟶

Stress adaptation ⟶ Calcipressin1

Cytochrome c/caspase 3 ⟶ Apoptosis (neuronal cells)

Vacuolar H$^+$/ Ca^{2+} exchangers ⟶ Ca^{2+} intolerance (yeast)

Plasma membrane Na$^+$/Ca^{2+} exchangers ⟶ Ca^{2+} accumulation (neuronal cells)
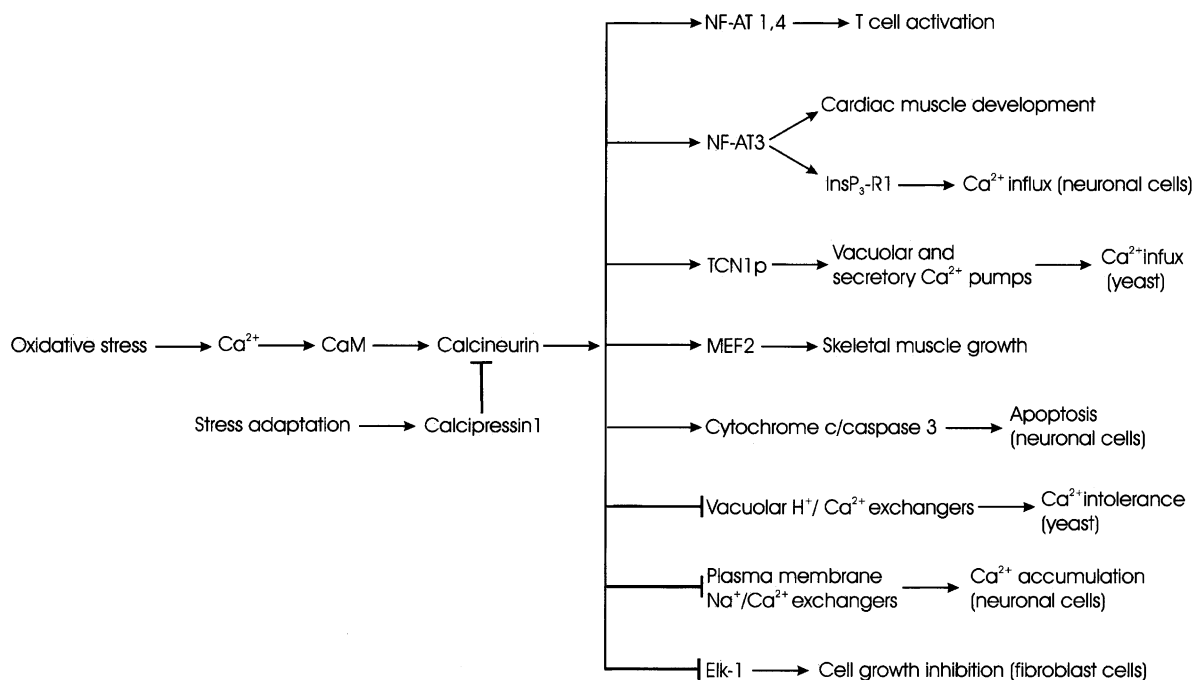
Elk-1 ⟶ Cell growth inhibition (fibroblast cells)

Fig. 2. Calcineurin pathways and oxidative stress. Oxidative stress causes rising Ca^{2+} concentrations in the cytoplasm and the nucleus. Ca^{2+} activates CaM binding to calcineurin and enhances its phosphatase activity. Calcineurin can then dephosphorylate transcription factors (NF-ATs; TCN1p; MEF2; Elk-1) and stimulate or repress the transcription of various genes. It can cause repression of the vacuolar H$^+$/Ca^{2+} exchangers in yeast and the plasma membrane Na$^+$/Ca^{2+} exchangers in neuronal cells. It also can cause cytochrome c release and caspase 3 activation, leading to apoptosis. Calcineurin can be inhibited by calcipressin1, which can protect cells against acute oxidative stress.

signaling to signals for cell death during severe oxidative stress.

4. Nuclei

The question of Ca^{2+} control in the nucleus still remains open. The pores in the nuclear envelope are large enough to permit free flow of calcium ions, suggesting that Ca^{2+} concentration in the nucleus depends entirely on that in the cytoplasm. Some studies, however, show that the nuclear envelope can be an efficient Ca^{2+} filter (see Santella and Carafoli, 1997 for review). Furthermore Ca^{2+} pumps, as well as InsP$_3$ and ADP ribose-modulated channels in the nuclear envelope, are suggested as possible Ca^{2+} filters. Although hydrogen peroxide may enter nuclei, other extra- and intracellular oxidants probably do not directly reach nuclei and modulate their functions. It is most likely that many oxidant signals are transformed and communicated to the nucleus by other means, the most significant of which probably involve Ca^{2+} signals. Above we discussed the observation that oxidants can regulate Ca^{2+} transport in and out of the cytoplasm, as well as within the cytoplasm, using Ca^{2+} pools that are stored in the ER/SR. Once the "Ca^{2+} code" is modulated in the cytoplasm it can also be modulated in the nucleus.

Some of the well known nuclear processes that are controlled by Ca^{2+} include fragmentation of nuclear proteins and intranucleosomal cleavage of chromatin during apoptosis by endonucleases, one of which is NUC18, and proteases such as a nuclear scaffold-associated protease (CRP) and calpain. NUC18 is an endonuclease with intranucleasomal activity that pre-exists in a repressed state in non-apoptotic cells, and becomes derepressed during apoptosis to digest chromosomal DNA and produce nucleosome-sized 180–200 base-pair fragments not seen in normal cells (Gaido and Cidlowski, 1991; Zhivotovsky et al., 1994). CRP and calpain activation are also associated with apoptosis. CRP cleaves lamins during apoptosis and promotes chromatin cleavage (Zhivotovsky et al., 1997). Increased calpain activity which precedes DNA fragmentation and morphological changes has been observed in several apoptotic models (Squier et al., 1994). Calpain is a heterodimeric cysteine protease consisting of two subunits: a catalytic subunit with homology to calmodulin, and a small regulatory subunit (Saido et al., 1994). Calpain has a number of substrates in the nucleus, including cytoskeletal proteins, calcineurin and PKC (Traub et al., 1988; Takahashi, 1990; Tallant et al., 1988), however, their cleavage by calpain seems to be Ca^{2+} independent.

In the nucleus Ca^{2+} can modulate the transcription of number of genes, including those encoding for: c-fos (Sheng

et al., 1990), various interleukins (Tsuboi et al., 1994), nerve growth factors (Enslen and Soderling, 1994), and others. In many cases it has been demonstrated that this process is mediated by transcription factors. Calcium can modulate the activity of kinases and phosphotases which, in turn can regulate the binding of transcription factors to promoters, and modulate gene transcription as a result. For example, Ca^{2+} has been demonstrated to activate CaM kinase that phosphorylates and activates the transcription factor CREB, that in turn leads to stimulation of c-fos expression (Sheng et al., 1990). Alternatively, the Ca^{2+}-stimulated phosphatase, calcineurin can dephosphorylate various transcription factors and modulate expression of a number of genes and cell functions. We will discuss this below in more detail.

5. Protein phosphorylation

Phosphorylation is an important regulator of many protein activities and signal transduction pathways. Phosphorylation/dephosphorylation reactions occur in both the nucleus and the cytoplasm, and they are controlled by protein kinases and phosphotases. It has been estimated that the mammalian genome might encode as many as 2000 distinct protein kinases and 1000 phosphotases (Hunter, 1995). Many of these enzymes can be controlled by calmodulin (CaM). When free Ca^{2+} in the nucleus rises, it binds and activates CaM, which consequently modulates the activity of protein kinases and phosphotases. One of the first described CaM-dependent kinases was myosin light-chain kinase (MLCK) (Simmen et al., 1984). This kinase has been detected in various cell types (Simmen et al., 1984; Bachs et al., 1990; Pujol et al., 1993), and it appears to have a range of functions: from a structural scaffold within the nucleus (Clark and Rosenbaum, 1979) and a molecular motor that controls chromatin movement (Billia and de Boni, 1991), to regulation of gene transcription (Egly et al., 1984), nuclear-cytoplasmic transport (Schindler and Jiang, 1986, 1990), and RNA processing by small heterogeneous ribonucleoprotein particles (Sahlas et al., 1993).

CaM kinases are also Ca^{2+}-dependent. They have been demonstrated to play important roles in cell cycle processes (Planas-Silva and Means, 1992; Lu and Means, 1993; Lorca et al., 1993; Deguchi and Osanai, 1994). A number of CaM kinases have been found in various cell types and the best characterized are different isoforms of CaM kinase II (see Santella and Carafoli, 1997 for review). CaM II has been shown to phosphorylate and modulate the activity of transcription factors, one of which is factor CREB that binds to cAMP response element.

Protein kinase C (PKC) can also be activated by rising Ca^{2+} concentrations in the nucleus (Tallant et al., 1988). PKC is involved in the regulation of cell proliferation and differentiation (Clemens et al., 1992). It has a number of substrates in the nucleus, one of which is the $InsP_3$ receptor (Matter et al., 1993). The phosphorylation of this receptor

leads to the release of Ca^{2+} to the perinuclear space, which might be a mechanism to minimize the rise in Ca^{2+} concentrations that accompany stress. Another type of kinase, mitogen-activated protein (MAP) kinase, can be activated by hydrogen peroxide (Guyton et al., 1996), however, it is not clear if this activation involves Ca^{2+}.

Protein phosphatases are a class of enzymes that antagonize the action of kinases. Calcineurin is the best characterized Ca^{2+}-dependent phosphatase. It is a calcium/calmodulin activated serine/threonine phosphatase (PP2B) that is an important enzyme in Ca^{2+}-dependent eukaryotic signal transduction pathways (Liu et al., 1991) and it has been detected in both the nucleus and the cytoplasm of various cell types. Calcineurin plays important roles in immune-stimulation, and calcineurin-dependent signal transduction pathways have been extensively characterized during T-cell activation (Clipstone and Crabtree, 1992). The general mechanism of T-cell activation includes Ca^{2+}-activated binding of CaM to calcineurin which enhances calcineurin's phosphatase activity. Calcineurin then dephosphorylates transcription factors such as NF-ATs and facilitates their transfer to the nucleus, where they bind to DNA and modulate gene expression (see Fig. 2). Transcription factors NF-AT 1 and 4 induce genes that activate T cells (Clipstone and Crabtree, 1992), while activation of NF-AT 3 causes heart hypertrophy (Molkentin et al., 1998). In yeast calcineurin has been shown to activate transcription factor TCN1p (Crz1p), which is homological to mammalian NF-ATs, and initiate Ca^{2+} release by vacuolar and secretory pumps, causing Ca^{2+} influx into the cytoplasm (Stathopoulos-Gerontides et al., 1999). It can activate factor MEF2 and stimulate skeletal muscle growth and differentiation (Wu et al., 2000; Chin et al., 1998; Hughes, 1998). It has also been demonstrated that calcineurin can antagonize the action of MAP kinases, and activate the transcription factor Elk-1 which regulates gene expression in response to a wide variety of extracellular stimuli (Sugimoto et al., 1997). Calcineurin plays a critical role in cellular responses to various extracellular signals and environmental stresses, and it is important in the regulation of apoptosis (Shibasaki and McKeon, 1995; Krebs, 1998; Asai et al., 1999), memory processes (Zhuo et al., 1999; Mansuy et al., 1998; Winder et al., 1998) and skeletal and cardiac muscle growth and differentiation (Wu et al., 2000; Chin et al., 1998; Molkentin et al., 1998). In plants calcineurin mediates salt adaptation (Pardo et al., 1998).

Calcineurin activity is sensitive to cellular redox state (Wang et al., 1996; Furuke et al., 1999; Reiter et al., 1999). For example, hydrogen peroxide can completely abrogate calcineurin-mediated NF-AT transactivation in response to stimulation (Reiter et al., 1999). It has also been shown that calcineurin inhibitors protect against Ca^{2+} paradox-induced cell toxicity, which is observed when cells are transferred from media without Ca^{2+} to media containing physiological Ca^{2+} concentrations (Takuma et al., 1999). These observations link calcineurin with both oxidative stress and

Ca^{2+}-signaling. The mechanism by which calcineurin mediates the interaction of oxidative stress with Ca^{2+} signaling is not yet understood. It might involve Ca^{2+} transport through $InsP_3$-sensitive channels in the ER/SR. Using a neuronal model it has been demonstrated that transcription of a major determinant of intracellular Ca^{2+}, $InsP_3$ receptor type 1 ($InsP_3$-R1), can be inhibited by FK506 and CsA calcineurin inhibitors (Graef et al., 1999). Using also a neuronal model it has recently been shown that calcineurin can inactivate the Na^+/Ca^{2+} exchanger plasma membrane NCX2 which exports Ca^{2+} from the cytosol to the extracellular space (Li et al., 2000). These and other data (see Fig. 2) suggest that activation of calcineurin by Ca^{2+} can cause further Ca^{2+} influx into the cytoplasm.

The recently discovered DSCR1 (Adapt78) encoded protein (calcipressin1) can bind to calcineurin and inhibit its activity (Fuentes et al., 2000; Rothermel et al., 2000). Expression of DSCR1 (Adapt78) is Ca^{2+}-dependent: it can be induced by calcium ionophores such as A23187, or other agents that increase intracellular Ca^{2+} concentrations. In contrast, cell-permeate calcium chelators, such BAPTA-AM, can block DSCR1 (Adapt78) expression (Crawford et al., 1997; Leahy et al., 1999). Using a neuronal cell model (PC-12 cells) we have recently demonstrated that calcipressin1 has a protective function against stress induced by either hydrogen peroxide or calcium (Davies et al., 2001). Calcipressin1 seems to protect cells against stress by binding to and downregulating the activity of calcineurin. Calcium concentrations during stress start rising in the cytoplasm and the nucleus. This causes activation of calcineurin which can have a damaging effect on cells (Asai et al., 1999), and acute calcipressin1 expression in this case can protect cells by downregulating calcineurin activity. Unfortunately, although short-term, stress-related, expression of the DSCR1 (Adapt78) gene appears to be protective, chronically high level expression is associated with Alzheimer's disease and Down syndrome (Ermak et al., 2001). The association of chronic DSCR1 (Adapt78) expression with Alzheimer's disease may involve hyperphosphorylation of the tau protein due to chronic inhibition of calcineurin; which otherwise would catalyze its dephosphorylation (Ermak et al., 2001). It is also possible that calcineurin antagonizes the essential activity of several stress-protective kinases. In this model the DSCR1 (Adapt78) gene product, calcipressin1, may protect cells by preventing calcineurin from inhibiting essential kinases (Davies et al., 2001).

Calcineurin transduction pathways are well characterized in yeast, where the enzyme promotes growth in high calcium environments by dephosphorylation of the Tcn1p/Crz1p transcription factor (Cunningham and Fink, 1994; Matheos et al., 1997; Stathopoulos-Gerontides et al., 1999). Also in yeast, calcineurin has been demonstrated to inhibit VCX1-dependent H^+/Ca^{2+} exchange by posttranslational mechanism and to induce Ca^{2+} ATPases (Cunningham and Fink, 1996), which can cause higher intracellular concentrations of Ca^{2+}. Mutants deleted in either Pmc1p or Pmr1p Ca^{2+} ATPases which are responsible for depleting the cytosol of Ca^{2+}, cannot grow in media containing high Ca^{2+} (Cunningham and Fink, 1994). However, deletion of the calcineurin gene, or treatment of cells with calcineurin inhibitor CsA or FK506, restores yeast growth, indicating that high calcineurin activity can have a negative effect on growth. This is in agreement with the finding that high levels of calcineurin activity predisposes neuronal cells to apoptosis, and this also supports our hypothesis that DSCR1 (Adapt78) expression can protect cells against stress by decreasing of calcineurin activity (Ermak et al., 2001; Davies et al., 2001).

6. Differential effects of oxidants on calcium signaling

The effect of oxidants on calcium signaling can vary from stimulative to repressive, depending on the type of oxidants, their concentrations, and the duration of exposure. For example, although most literature data suggest that oxidants enhance calcium flow through $InsP_3$-gated channels, in some studies this effect was different. In a study by Elliott and Doan (1993), oxidant stress induced by t-butyl hydroperoxide inhibited bradykinin-stimulated Ca^{2+} signaling in vascular endothelial cells. $InsP_3$-sensitive Ca^{2+} pools in this case remained replete and releasable, and intracellular Ca^{2+} release in contrast was attenuated. Another study found that hydrogen peroxide blocks thrombin- and histamine-stimulated $InsP_3$ expression in vascular endothelial cells (Vercellotti et al., 1991). This may be due to the concentration of peroxides used in these studies, which were higher than in the studies in which $InsP_3$-mediated calcium transport was increased, and it is likely that modulation of $InsP_3$-gated calcium channels is dependent on oxidant concentrations.

Superoxide anions and peroxide can differentially modulate $InsP_3$-sensitive and -insensitive pools. In a study by Elmoselhi et al. (1996) on smooth muscle cells, it was demonstrated that superoxide anions only inhibit $InsP_3$-sensitive channels, whereas hydrogen peroxide inhibits both $InsP_3$-sensitive and -insensitive ones. Oxidant concentrations and duration of exposure could dramatically change calcium signaling and even reverse its direction from stimulative to repressive (or the other way around). In human aortic endothelial cells low concentrations (1–10 μM) of hydrogen peroxide exhibit no effect on Ca^{2+}, while 100 μM hydrogen peroxide concentration induced intracellular calcium oscillations (Hu et al., 1998). After reaching a peak these oscillations remained above baseline for some time before gradually returning to normal.

It is interesting that Ca^{2+} activation pathways depend not only on the magnitude and duration of Ca^{2+} influxes, but also on the types of cell domains in which they occur. For example, intracellular Ca^{2+} entry into neuronal cells via voltage-activated (e.g. the L-type channels) and ligand-activated channels (present in glutamate receptors

activated by the antagonist-methyl-aspartame; the NMDA channels) initiate different gene transcription pathways (see Gallin and Greenberg, 1995). Voltage-activated channels are localized in the cell body and the proximal portion of dendrites, whereas NMDA channels are in the postsynaptic regions of dendrites. Calcium influx through either channel activates CaM kinases, but the consequences of such activation through NMDA channels are unknown, while influx through voltage-activated channels causes phosphorylation of the transcription factor CREB, which facilitates its binding to the CaRE/CRE DNA motif and initiates c-fos transcription. In voltage-activated channels CaM kinase also stimulates ERF-transcription factor binding to ERE, while in NMDA channels this process does not directly involve CaM. In addition, in cells stimulated via voltage-activated channels CaRE/CRE- and ERE-elements can operate independently, while in NMDA-stimulated cells they have to co-operate to achieve c-fos activation.

7. Discussion

Here we see that various oxidants cause Ca^{2+} influx into the cytoplasm, and consequently into both the mitochondria and the nucleus. Calcium can be used then as a signal molecule to respond to oxidant stimuli. It can activate and repress proteins, and gene transcription. Generally oxidants activate Ca^{2+} channels, repress pumps, and can reverse Na^+/Ca^{2+} exchangers. However, the mechanism by which oxidants exert such regulation are poorly understood. It is possible that some channels, pumps, or exchangers can be directly oxidized and modulated, as was described above for Ca^{2+} ATPase pumps.

Oxidation of the Ca^{2+} ATPases inhibits their ability to extrude Ca^{2+} out of the cell or inside the ER/SR, causing Ca^{2+} concentrations in the cytoplasm to rise. In addition, regulatory molecules that modulate pump activity can also be oxidized and loose their regulatory properties. Indeed, it has been shown that during the aging process, which is associated with oxidative stress, multiple methionines within the CaM are oxidized, resulting in its inability to activate a range of target proteins, including the plasma membrane and the ER/SR Ca^{2+}-ATPases involved in the maintenance of low cytoplasmic Ca^{2+} concentrations (Squier and Bigelow, 2000). However, if vital protein molecules that form channels are also oxidized, it would disrupt their function, resulting in a lower Ca^{2+} concentration in the cytoplasm. Unexpectedly, a number of studies indicate that the functions of channels are stimulated but not inhibited by the oxidants causing additional rising Ca^{2+} concentration in the cytoplasm (e.g. Renard et al., 1992; Missiaen et al., 1991; Henschke and Elliott, 1995). This has been shown to be caused by the formation of mixed disulfides with GSSG (oxidized glutathione) or other oxidized thiols that can interact with, and open $InsP_3$ channels. Surprisingly, not only Ca^{2+} pumps or channels are responsible for Ca^{2+}

transport during stress conditions. For example, hydrogen peroxide can cause Ca^{2+} influx in neuronal-like PC-12 cells through both calcium and sodium channels (Wang and Joseph, 2000). And actually, sodium channels appear to be more effective than calcium channels for hydrogen peroxide induced Ca^{2+} influx.

Protein kinases and phosphatases are vital for the transduction of various extra- and intracellular signals, presumably including oxidant signals. As discussed above, at this time only one phosphatase and several kinases out of the possible thousands have been linked to oxidative stress and Ca^{2+} signaling. These facts indicate that we are now taking only the first step in understanding how oxidants modulate Ca^{2+} signaling.

References

Asai, A., Qui, J., Narita, Y., et al., 1999. High level calcineurin activity predisposes neuronal cells to apoptosis. J. Biol. Chem. 274, 34450–34458.

Bachs, O., Lanini, L., Serratosa, J., Coll, M.J., Bastos, R., Aligue, R., Rius, E., Carafoli, E., 1990. Calmodulin-binding proteins in the nuclei of quiescent and proliferatively activated rat liver cells. J. Biol. Chem. 265, 18595–18600.

Berridge, M.J., Bootman, M.D., Lipp, P., 1998. Calcium—a life and death signal. Nature 395, 645–648.

Billia, F., de Boni, U., 1991. Localization of centromeric satellite and telomeric DNA sequences in dorsal root ganglion neurons, in vitro. J. Cell Sci. 100, 219–226.

Castilho, R.F., Carvalho-Alves, P.C., Vercesi, A.E., Ferreira, S.T., 1996. Oxidative damage to sarcoplasmic reticulum Ca$^{(2+)}$-pump induced by Fe^{2+}/H$_2$O$_2$/ascorbate is not mediated by lipid peroxidation or thiol oxidation and leads to protein fragmentation. Mol. Cell Biochem. 159, 105–114.

Chin, E.R., Olson, E.N., Richardson, J.A., et al., 1998. A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. Genes Dev. 12, 2499–2509.

Clague, J.R., Langer, G.A., 1994. The pathogenesis of free radical-induced calcium leak in cultured rat cardiomyocytes. J. Mol. Cell Cardiol. 26, 11–21.

Clark, T.G., Rosenbaum, J.L., 1979. An actin filament matrix in hand-isolated nuclei of *X. laevis* oocytes. Cell 18, 1101–1108.

Clemens, M.J., Trayner, I., Menaya, J., 1992. The role of protein kinase C isoenzymes in the regulation of cell proliferation and differentiation. J. Cell Sci. 103, 881–887.

Clipstone, N.A., Crabtree, G.R., 1992. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 357, 695–697.

Crawford, D.R., Leahy, K.P., Abramova, N., Lan, L., Wang, Y., Davies, K.J.A., 1997. Hamster adapt78 mRNA is a down syndrome critical region homologue that is inducible by oxidative stress. Arch. Biochem. Biophys. 342, 6–12.

Cunningham, K.W., Fink, G.R., 1994. Calcineurin-dependent growth control in *Saccharomyces cerevisiae* mutants lacking PMC1, a homolog of plasma membrane Ca^{2+} ATPases. J. Cell Biol. 124, 351–363.

Cunningham, K.W., Fink, G.R., 1996. Calcineurin inhibits VCX1-dependent H$^+$/Ca^{2+} exchange and induces Ca^{2+} ATPases in *Saccharomyces cerevisiae*. Mol. Cell Biol. 16, 2226–2237.

Davies, K.J.A., 1999. The broad spectrum of responses to oxidants in proliferating cells: a new paradigm for oxidative stress. IUBMB Life 48, 1–7.

Davies, K.J.A., 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50, 279–289.

Davies, K.J.A., Harris, C.D., Ermak, G., 2001. The essential role of calcium in induction of the *DSCR1* (*Adapt78*) gene. BioFactors 12, 1–3.

Deguchi, R., Osanai, K., 1994. Repetitive intracellular Ca^{2+} increases at fertilization and the role of Ca^{2+} in meiosis reinitiation from the first metaphase in oocytes of marine bivalves. Dev. Biol. 163, 162–174.

Doan, T.N., Gentry, D.L., Taylor, A.A., Elliott, S.J., 1994. Hydrogen peroxide activates agonist-sensitive $Ca^{(2+)}$-flux pathways in canine venous endothelial cells. Biochem. J. 297, 209–215.

Egly, J.M., Miyamoto, N.G., Moncollin, V., Chambon, P., 1984. Is actin a transcription initiation factor for RNA polymerase B? EMBO J. 3, 2363–2371.

Elliott, S.J., Doan, T.N., 1993. Oxidant stress inhibits the store-dependent $Ca^{(2+)}$-influx pathway of vascular endothelial cells. Biochem. J. 292, 385–393.

Elliott, S.J., Meszaros, J.G., Schilling, W.P., 1992. Effect of oxidant stress on calcium signaling in vascular endothelial cells. Free Rad. Biol. Med. 13, 635–650.

Elmoselhi, A.B., Samson, S.E., Grover, A.K., 1996. ER/SR Ca^{2+} pump heterogeneity in coronary artery: free radicals and IP_3-sensitive and -insensitive pools. Am. J. Physiol. 271, C1652–C1659.

Enslen, H., Soderling, T.R., 1994. Roles of calmodulin-dependent protein kinases and phosphatase in calcium-dependent transcription of immediate early genes. J. Biol. Chem. 269, 20872–20877.

Ermak, G., Morgan, T.E., Davies, K.J.A., 2001. Chronic overexpression of the calcineurin inhibitory gene *DSCR1* (*Adapt78*) is associated with Alzheimer disease. J. Biol. Chem. 42, 38787–38794.

Fuentes, J.J., Genesca, L., Kingsbury, T.J., et al., 2000. DSCR1, overexpressed in down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. Hum. Mol. Genet. 9, 1681–1690.

Furuke, K., Shiraishi, M., Mostowski, H.S., Bloom, E.T., 1999. Fas ligand induction in human NK cells is regulated by redox through a calcineurin-nuclear factors of activated T cell-dependent pathway. J. Immunol. 162, 1988–1993.

Gaido, M.L., Cidlowski, J.A., 1991. Identification, purification, and characterization of a calcium-dependent endonuclease (NUC18) from apoptotic rat thymocytes. NUC18 is not histone H2B. J. Biol. Chem. 266, 18580–18585.

Gallin, W.J., Greenberg, M.E., 1995. Calcium regulation of gene expression in neurons: the mode of entry matters. Curr. Opin. Neurobiol. 5, 367–374.

Graef, I.A., Mermelstein, P.G., Stankunas, K., Neilson, J.R., Deisseroth, K., Tsien, R.W., Crabtree, G.R., 1999. L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. Nature 401, 703–708.

Graier, W.F., Hoebel, B.G., Paltauf-Doburzynska, J., Kostner, G.M., 1998. Effects of superoxide anions on endothelial Ca^{2+} signaling pathways. Arterioscl. Thromb. Vasc. Biol. 18, 1470–1479.

Griffiths, E.J., Ocampo, C.J., Savage, J.S., Rutter, G.A., Hansford, R.G., Stern, M.D., Silverman, H.S., 1998. Mitochondrial calcium transporting pathways during hypoxia and reoxygenation in single rat cardiomyocytes. Cardiovasc. Res. 39, 423–433.

Grover, A.K., Samson, S.E., 1988. Effect of superoxide radical on Ca^{2+} pumps of coronary artery. Am. J. Physiol. 255, C297–C303.

Grover, A.K., Samson, S.E., Fomin, V.P., 1992. Peroxide inactivates calcium pumps in pig coronary artery. Am. J. Physiol. 263, H537–H543.

Gunter, T.E., Buntinas, L., Sparagna, G.C., Gunter, K.K., 1998. The Ca^{2+} transport mechanisms of mitochondria and Ca^{2+} uptake from physiological-type Ca^{2+} transients. Biochim. Biophys. Acta 1366, 5–15.

Guyton, K.Z., Liu, Y., Xu, M., Gorospe, Q., Holbrook, N.J., 1996. Activation of mitogen-activated protein kinase by H_2O_2, role in cell survival following oxidant injury. J. Biol. Chem. 271, 4138–4142.

Henschke, P.N., Elliott, S.J., 1995. Oxidized glutathione decreases luminal Ca^{2+} content of the endothelial cell ins(1,4,5)P_3-sensitive Ca^{2+} store. Biochem. J. 312, 485–489.

Hu, Q., Corda, S., Zweier, J.L., Capogrossi, M.C., Ziegelstein, R.C., 1998. Hydrogen peroxide induces intracellular calcium oscillations in human aortic endothelial cells. Circulation 97, 268–275.

Hughes, S.M., 1998. Muscle development: electrical control of gene expression. Curr. Biol. 8, 892–894.

Hunter, T., 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 80, 225–236.

Joseph, J.A., Strain, J.G., Jimenez, N.D., Fisher, D., 1997. Oxidant injury in PC12 cells—a possible model of calcium "dyERegulation" in aging. I. Selectivity of protection against oxidative stress. J. Neurochem. 69, 1252–1258.

Kaneko, M., Matsumoto, Y., Hayashi, H., Kobayashi, A., Yamazaki, N., 1994. Oxygen free radicals and calcium homeostasis in the heart. Mol. Cell Biochem. 135, 99–108.

Krebs, J., 1998. The role of calcium in apoptosis. Biometals 11, 375–382.

Lander, H.M., 1997. An essential role for free radicals and derived species in signal transduction. FASEB J. 11, 118–124.

Leahy, K.P., Davies, K.J., Dull, M., Kort, J.J., Lawrence, K.W., Crawford, D.R., 1999. Adapt78, a stress-inducible mRNA, is related to the glucose-regulated protein family of genes. Arch. Biochem. Biophys. 368, 67–74.

Li, L., Guerini, D., Carafoli, E., 2000. Calcineurin controls the transcription of Na^+/Ca^{2+} exchanger isoforms in developing cerebellar neurons. J. Biol. Chem. 275, 20903–20910.

Liu, J., Farmer, J.D., Lane, W.S., et al., 1991. Calcineurin is a common target of cyclosporin–cyclosporin A and FKBP–FK506 complexes. Cell 66, 807–815.

Lorca, T., Cruzalegui, F.H., Fesquet, D., Cavadore, J.C., Mery, J., Means, A., Doree, M., 1993. Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of xenopus eggs. Nature 366, 270–273.

Lu, K.P., Means, A.R., 1993. Regulation of the cell cycle by calcium and calmodulin. Endocr. Rev. 14, 40–58.

Lynch, D.R., Dawson, T.M., 1994. Secondary mechanisms in neuronal trauma. Curr. Opin. Neurol. 7, 510–516.

Mansuy, I.M., Mayford, M., Jacob, B., Kandel, E.R., Bach, M.E., 1998. Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. Cell 92, 39–49.

Marin, J., Encabo, A., Briones, A., Garcia-Cohen, E.C., Alonso, M.J., 1999. Mechanisms involved in the cellular calcium homeostasis in vascular smooth muscle: calcium pumps. Life Sci. 64, 279–303.

Matheos, D.P., Kingsbury, T.J., Ahsan, U.S., Cuningham, K.W., 1997. Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in *Saccharomyces cerevisiae*. Genes Dev. 11, 3445–3458.

Matter, N., Ritz, M.F., Freyermuth, S., Rogue, P., Malviya, A.N., 1993. Stimulation of nuclear protein kinase C leads to phosphorylation of nuclear inositol 1,4,5-trisphosphate receptor and accelerated calcium release by inositol 1,4,5-trisphosphate from isolated rat liver nuclei. J. Biol. Chem. 268, 732–736.

Missiaen, L., Taylor, C.W., Berridge, M.J., 1991. Spontaneous calcium release from inositol trisphosphate-sensitive calcium stores. Nature 352, 241–244.

Molkentin, J.D., Lu, J.R., Antos, C.L., Markham, B., Richardson, J., Robbins, J., Grant, S.R., Olson, E.N., 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 93, 215–228.

Pacifici, R.E., Davies, K.J.A., 1991. Protein, lipid and DNA repair systems in oxidative stress: the free-radical theory of aging revisited. Gerontology 37, 166–180.

Pardo, J.M., Reddy, M.P., Yang, S., et al., 1998. Stress signaling through Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants. Proc. Natl. Acad. Sci. U.S.A. 95, 9681–9686.

Planas-Silva, M.D., Means, A.R., 1992. Expression of a constitutive form of calcium/calmodulin dependent protein kinase II leads to arrest of the cell cycle in G2. EMBO J. 11, 507–517.

Pujol, M.J., Bosser, R., Vendrell, M., Serratosa, J., Bachs, O., 1993. Nuclear calmodulin-binding proteins in rat neurons. J. Neurochem. 60, 1422–1428.

Reiter, T.A., Abraham, R.T., Choi, M., Rusnak, F., 1999. Redox regulation of calcineurin in T-lymphocytes. J. Biol. Inorg. Chem. 4, 632–644.

Renard, D.C., Seitz, M.B., Thomas, A.P., 1992. Oxidized glutathione causes sensitization of calcium release to inositol 1,4,5-trisphosphate in permeabilized hepatocytes. Biochem. J. 284, 507–512.

Rizzuto, R., Pinton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.M., Tuft, R.A., Pozzan, T., 1998. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science 280, 1763–1766.

Rizzuto, R., Bernardi, P., Pozzan, T., 2000. Mitochondria as all-round players of the calcium game. J. Physiol. 529 (1), 37–47.

Rohn, T.T., Hinds, T.R., Vincenzi, F.F., 1993. Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca^{2+} pump ATPase and Na^{+}/K^{+} pump ATPase of human red blood cell membranes. Biochem. Pharmacol. 46, 525–534.

Rooney, T.A., Renard, D.C., Sass, E.J., Thomas, A.P., 1991. Oscillatory cytosolic calcium waves independent of stimulated inositol 1,4,5-trisphosphate formation in hepatocytes. J. Biol. Chem. 266, 12272–12282.

Rothermel, B., Vega, R.B., Yang, J., Wu, H., Bassel-Duby, R., Williams, R.S., 2000. A protein encoded within the down syndrome critical region is enriched in striated muscles and inhibits calcineurin signaling. J. Biol. Chem. 275, 8719–8725.

Roveri, A., Coassin, M., Maiorino, M., Zamburlini, A., van Amsterdam, F.T., Ratti, E., Ursini, F., 1992. Effect of hydrogen peroxide on calcium homeostasis in smooth muscle cells. Arch. Biochem. Biophys. 297, 265–270.

Sahlas, D.J., Milankov, K., Park, P.C., De Boni, U., 1993. Distribution of snRNPs, splicing factor SC-35 and actin in interphase nuclei: immunocytochemical evidence for differential distribution during changes in functional states. J. Cell Sci. 105, 347–357.

Saido, T.C., Sorimachi, H., Suzuki, K., 1994. Calpain: new perspectives in molecular diversity and physiological–pathological involvement. FASEB J. 8, 814–822.

Santella, L., Carafoli, E., 1997. Calcium signaling in the cell nucleus. FASEB J. 11, 1091–1109.

Scherer, N.M., Deamer, D.W., 1986. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca^{2+}-ATPase. Arch. Biochem. Biophys. 246, 589–601.

Schindler, M., Jiang, L.W., 1986. Nuclear actin and myosin as control elements in nucleocytoplasmic transport. J. Cell Biol. 102, 859–862.

Schindler, M., Jiang, L.W., 1990. A dynamic analysis of nuclear pore complex—the intelligent tunel. In: Wang, E., Wang, J.L., Cheung, W.Y., Wu, C.W. (Eds.), Biochemical and Structural Dynamics of the Cell Nucleus. Academic Press, San Diego, CA, pp. 249–263.

Sheng, M., McFadden, G., Greenberg, M.E., 1990. Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. Neuron 4, 571–582.

Shibasaki, F., McKeon, F., 1995. Calcineurin functions in $Ca(2+)$-activated cell death in mammalian cells. J. Cell Biol. 131, 735–743.

Simmen, R.C., Dunbar, B.S., Guerriero, V., Chafouleas, J.G., Clark, J.H., Means, A.R., 1984. Estrogen stimulates the transient association of calmodulin and myosin light chain kinase with the chicken liver nuclear matrix. J. Cell Biol. 99, 588–593.

Squier, T.C., Bigelow, D.J., 2000. Protein oxidation and age-dependent alterations in calcium homeostasis. Front. Biosci. 5, D504–526.

Squier, M.K., Miller, A.C., Malkinson, A.M., Cohen, J.J., 1994. Calpain activation in apoptosis. J. Cell Physiol. 159, 229–237.

Stathopoulos-Gerontides, A., Guo, J.J., Cyert, M.S., 1999. Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev. 13, 798–803.

Stout, A.K., Raphael, H.M., Kanterewicz, B.I., Klann, E., Reynolds, I.J., 1998. Glutamate-induced neuron death requires mitochondrial calcium uptake. Nat. Neurosci. 1, 366–373.

Sugimoto, T., Stewart, S., Guang, K., 1997. The calcium/calmodulin-dependent protein phosphatase calcineurin is the major Elk-1 phosphatase. J. Biol. Chem. 272, 29415–29418.

Suzuki, Y.J., Ford, G.D., 1991. Inhibition of $Ca(2+)$-ATPase of vascular smooth muscle sarcoplasmic reticulum by reactive oxygen intermediates. Am. J. Physiol. 261, H568–H574.

Takahashi, K., 1990. Calpain substrate specifity. In: Melgren, R.L., Murachi, T. (Eds.), Intracellular Calcium-Dependent Proteolysis. CRC Press, Boca Raton, FL, pp. 55–74.

Takuma, K., Lee, E., Kidawara, M., Mori, K., Kimura, Y., Baba, A., Matsuda, T., 1999. Apoptosis in Ca^{2+} reperfusion injury of cultured astrocytes: roles of reactive oxygen species and NF-kappaB activation. Eur. J. Neurosci. 11, 4204–4212.

Tallant, E.A., Brumley, L.M., Wallace, R.W., 1988. Activation of a calmodulin-dependent phosphatase by a Ca^{2+}-dependent protease. Biochemistry 27, 2205–2211.

Traub, P., Scherbarth, A., Willingale-Theune, J., Paulin-Levasseur, M., Shoeman, R., 1988. Differential sensitivity of vimentin and nuclear lamins from Ehrlich ascites tumor cells toward Ca^{2+}-activated neutral thiol proteinase. Eur. J. Cell Biol. 46, 478–490.

Tsuboi, A., Muramatsu, M., Tsutsumi, A., Arai, K., Arai, N., 1994. Calcineurin activates transcription from the GM-CSF promoter in synergy with either protein kinase C or NF-kappa B/AP-1 in T cells. Biochem. Biophys. Res. Commun. 199, 1064–1072.

Vercellotti, G.M., Severson, S.P., Duane, P., Moldow, C.F., 1991. Hydrogen peroxide alters signal transduction in human endothelial cells. J. Lab. Clin. Med. 117, 15–24.

Wang, H., Joseph, J.A., 2000. Mechanism of hydrogen peroxide-induced calcium deregulation in PC12 cells. Free Rad. Biol. Med. 28, 1222–1231.

Wang, X., Culotta, V.C., Klee, C.B., 1996. Superoxide dismutase protects calcineurin from inactivation. Nature 383, 434–437.

Winder, D.G., Mansuy, I.M., Osman, M., Moallem, T.M., Kandel, E.R., 1998. Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. Cell 92, 25–37.

Wu, H., Naya, F.J., McKinsey, T.A., Mercer, B., Shelton, J.M., Chin, E.R., Simard, A.R., Michel, R.N., Bassel-Duby, R., Olson, E.N., Williams, R.S., 2000. MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. EMBO J. 19, 1963–1973.

Xu, K.Y., Zweier, J.L., Becker, L.C., 1997. Hydroxyl radical inhibits sarcoplasmic reticulum $Ca(2+)$-ATPase function by direct attack on the ATP binding site. Circ. Res. 80, 76–81.

Zhivotovsky, B., Wade, D., Gahm, A., Orrenius, S., Nicotera, P., 1994. Formation of 50 kbp chromatin fragments in isolated liver nuclei is mediated by protease and endonuclease activation. FEBS Lett. 351, 150–154.

Zhivotovsky, B., Gahm, A., Orrenius, S., 1997. Two different proteases are involved in the proteolysis of lamin during apoptosis. Biochem. Biophys. Res. Commun. 233, 96–101.

Zhuo, S., Zhang, W., Son, H., et al., 1999. A selective role of calcineurin Aα in synaptic depotentiation in hippocampus. Proc. Natl. Acad. Sci. U.S.A. 96, 4650–4655.