BioMetals **14:** 331–341, 2001.

Review

Functions of zinc in signaling, proliferation and differentiation of mammalian cells

Detmar Beyersmann* & Hajo Haase
*Department of Biology and Chemistry, University of Bremen, D-28334 Bremen, Germany; *Author for correspondence (Tel: +49-421-2182377; Fax: +49-421-2187433; E-mail: beyers@chemie.uni-bremen.de)*

Received 18 January 2001; accepted 17 May 2001

Key words: differentiation, metallothionein, proliferation, signal transduction, zinc

Abstract

Zinc is essential for cell proliferation and differentiation, especially for the regulation of DNA synthesis and mitosis. On the molecular level, it is a structural constituent of a great number of proteins, including enzymes of cellular signaling pathways and transcription factors. Zinc homeostasis in eukaryotic cells is controlled on the levels of uptake, intracellular sequestration in zinc storing vesicles ('zincosomes'), nucleocytoplasmic distribution and elimination. These processes involve the major zinc binding protein metallothionein as a tool for the regulation of the cellular zinc level and the nuclear translocation of zinc in the course of the cell cycle and differentiation. In addition, there is also increasing evidence for a direct signaling function for zinc on all levels of signal transduction. Zinc can modulate cellular signal recognition, second messenger metabolism, protein kinase and protein phosphatase activities, and it may stimulate or inhibit activities of transcription factors, depending on the experimental systems studied. Zinc has been shown to modify specifically the metabolism of cGMP, the activities of protein kinase C and mitogen activated protein kinases, and the activity of transcription factor MTF-1 which controls the transcription of the genes for metallothionein and the zinc transporter ZnT-1. As a conclusion of these observations new hypotheses regarding regulatory functions of zinc ions in cellular signaling pathways are proposed.

Abbreviations: ATF-2 – Activating transcription factor-2; CAF – CREB associated factor; cAMP – Cyclic adenosine monophosphate; CaMPK-2 – Calcium/calmodulin dependent protein kinase-2; cGMP – Cyclic guanosine monophosphate; CREB– Cyclic AMP response element binding factor; DTPA – Diethylenetriaminepentaacetic acid; EGF – Epidermal growth factor; ERK – Extracellular signal-regulated kinase; GABA – γ-Aminobutyric acid; IGF-1 – Insulin-like growth factor-1; JNK – Jun-kinase; MAPK – Mitogen activated protein kinase; MT – Metallothionein; MTF-1 – Metal response element-binding transcription factor-1; NMDA – N-Methyl-D-aspartic acid; NO – Nitric monoxide; PDE – Cyclic nucleotide phosphodiesterase; PI3K – Phosphatidylinositol 3-kinase; PKC – Protein kinase C; TPEN, N,N,N′,N′-Tetrakis(2-pyridylmethyl) ethylenediamine; ZnT-1 – Zinc transporter-1.

Introduction

The essentiality of zinc for growth and proliferation was recognized by the observation that zinc deficiency caused growth retardation in all organisms investigated (Vallee & Falchuk 1993). Growth and differentiation of eukaryotic cells generally are induced by growth hormones/growth factors that trigger cascades of intracellular signaling elements. These include hormone receptors, intracellular second messengers, cascades of protein kinases, protein phosphatases and transcription factors binding to promoters of the genes to be addressed. On all levels of cellular signal transduction zinc is involved, either as a structural element or a regulatory factor or both. Thus zinc is an essential prerequisite for the progress of many signaling processes in eukaryotes. But there is also evidence for a direct signaling function of zinc: It modulates

the GABA and NMDA receptors in mammalian brain cells (Cuajungco & Lees 1997; Canzoniero *et al.* 1997) and it binds to zinc sensing domains as in the case of the metal-regulated transcription factor MTF-1 (Andrews, this issue). Further, transcription factors have been detected that require zinc in the medium for binding to enhancers or their associated factors and probably are subject to modulation by zinc (Berg *et al.* 1997; Inada *et al.* 1997). All crucial decisions in the life of mammalian cells are involving zinc in its ionic or protein-bound form: be it cell growth and proliferation, differentiation or programmed cell death.

Intracellular distribution of zinc

A regulatory function of zinc requires a strict regulation of the cellular zinc content and its distribution. 30 to 40% of the cellular zinc is localized in the nucleus, 50% in the cytosol and cytosolic organelles and the remainder is associated with membranes (Vallee & Falchuk 1993). In the last few years, some of the zinc transport proteins that maintain this distribution have been identified in mammalian cells. These include plasma membrane zinc importer and zinc exporter proteins as well as transporters that mediate the sequestration of Zn^{2+} into intracellular vesicles. For a detailed discussion of zinc transporters, see Gaither & Eide (this issue).

Most of the cellular zinc is bound to or at least associated with proteins or complexed by anions, hence the level of free intracellular zinc is very low. The major Zn^{2+} binding protein in mammalian systems is metallothionein which donates Zn^{2+} to enzymes (Udom & Brady 1980, Jacob *et al.* 1998) and transcription factors with zinc finger domains (Zeng *et al.* 1991, Cano-Gauci & Sarkar 1996, Maret *et al.* 1997) wheras the apoprotein thionein accepts Zn^{2+} from binding sites in proteins with moderate affinity for Zn^{2+} (Maret *et al.* 1999). The latter authors have estimated from enzyme inhibition constants that inhibition of crucial enzymes by zinc may become significant with free Zn^{2+} above 10^{-8} M. Hence, the free cellular Zn^{2+} concentration may be estimated to be of this order of magnitude.

Several techniques have been used to determine the free zinc concentration experimentally. Measurements with radioactive ^{65}Zn yielded a concentration of 24 pM (Simons 1991), and ^{19}F-NMR spectroscopy with 5-F-BAPTA gave a concentration of 0.5 nM free intracellular zinc (Benters *et al.* 1997). With fluorescent probes traditionally employed for Ca^{2+} analysis, intracellular free Zn^{2+} concentrations were estimated to be 1 nM with FURA-2 (Atar *et al.* 1995) and 2 nM with Mag-Fura-2 (Sensi *et al.* 1997). Whereas these probes are not specific for Zn^{2+} and thus their signals are difficult to separate from those for Ca^{2+}, the zinc-specific fluorescent probe Zinquin allows a specific detection of intracellular zinc, but a quantitative analysis is hampered by the lack of a ratio-technique with the Zinquin spectrum. An estimation of cellular Zn^{2+} levels from the Zinquin fluorescence results in micromolar concentrations. These high values are at least in part due to a complexation of protein-bound zinc as has been shown for metallothionein (Coyle *et al.* 1994). Therefore Zinquin cannot be used for a quantification of free cytosolic zinc ions but it has been shown to be an excellent tool to investigate the intracellular distribution of loosely bound zinc, i.e. the amount of the ion that can be easily exchanged and therefore should be considered as the mediator of a signaling function for zinc. Recently, a new fluorescent Zn^{2+} chelator, Zinpyr-1, has been developed with an apparent dissociation constant of 2 nM (Walkup *et al.* 2000). However, this new probe like Zinquin does not allow the ratio-technique, which would allow precise quantification of intracellular Zn^{2+}.

In a number of cell lines it has been demonstrated with Zinquin that there is a typical pattern of zinc-specific fluorescence (Zalewski *et al.* 1993, Coyle *et al.* 1994). First it consists of a fluorescent cytosol, second a nearly nonfluorescent nucleus and third of zincosomes, vesicular structures containing high amounts of loosely bound zinc (Figure 1). Furthermore, the total cellular zinc level is tightly regulated. For example, in C6 rat glioma cells the total cellular zinc content is independent of the extracellular zinc concentration up to a threshold of 100 μM (Haase & Beyersmann 1999) mediated by an export mechanism for zinc that can be inhibited by lanthanum(III) ions in this cell line (Haase 2001). These observations demonstrate the existence of a complex regulation of the total zinc and its intracellular distribution, which both are a necessary basis for a function of zinc in signal transduction.

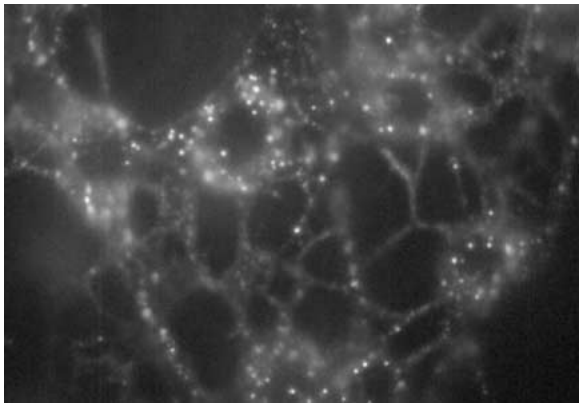

Figure 1. Intracellular distribution of loosely bound zinc ions. C6 rat glioma cells were loaded with the zinc-specific fluorescent probe Zinquin. Typical observations are a fluorescent cytosol, a nearly nonfluorescent nucleus and vesicular structures of high fluorescence intensity, so-called zincosomes. Magnification of original photograph is 400×.

Effect of zinc on major cellular signaling mechanisms

Zinc is involved in extracellular signal recognition, second messenger metabolism, protein phosphorylation and dephosphorylation and activity of transcription factors. In several instances, zinc ions have been shown to directly modulate cellular signaling. Figure 2 depicts the main interactions of zinc with receptors, protein kinases and transcription factors, which are described in detail below.

Calcium signaling

Zinc interferes with different aspects of calcium regulation. Electrical stimulation of heart cells evoked Zn^{2+} entry through voltage-dependent Ca^{2+} channels, and the addition of extracellular Zn^{2+} to spontaneously depolarizing pituitary tumor cells induced the expression of a reporter gene driven by the metallothionein promoter (Atar *et al.* 1995). In some cell types, elevation of extracellular zinc evoked intracellular Ca^{2+} mobilization. E.g., in primary hepatocyte cultures, 100 μM Zn^{2+} caused an increase in the intracellular free calcium concentration by stimulation of hormone sensitive intracellular calcium stores (McNulty & Taylor 1999). The authors of this report postulate the existence of a hepatic heavy metal stimulated receptor which is only expressed in hepatocytes and hypothesized that zinc might function as a local hormone that is secreted with insulin from pancreatic β-cells and participates in the regulation of liver metabolism. Employing the fluorescent probe Zinquin, a release of zinc from secretory vesicles of pancreatic islet cells was indeed shown to be induced by insulin secretagogues (Qian *et al.* 2000).

For the calcium/calmodulin-dependent protein kinase-2 (CaMPK-2), opposite effects of low and elevated zinc concentrations were observed. Whereas low zinc concentrations resulted in an increase of calmodulin-independent activity, high levels of zinc inhibited the binding of Ca^{2+}-calmodulin and the activity of the kinase (Lengyel *et al.* 2000).

Cyclic nucleotide metabolism

The cellular contents of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are regulated via their synthesis by cyclases and their degradation by cyclic nucleotide phosphodiesterases (PDE). Both groups of enzymes consist of large families of different subtypes. Zinc has been shown to affect these signal transduction pathways by modulating PDE activities. After the first observation of an inhibition of cAMP and cGMP hydrolysis by zinc in bovine heart (Donelly 1978), there have been several reports of both activating and inhibiting effects of zinc on different PDE subfamilies. Cyclic nucleotide phosphodiesterases are assumed to be zinc hydrolases, because two typical zinc-binding sequences ($HX_3HX_{24\text{-}26}E$) have been identified in the catalytic domain of PDE V (Francis *et al.* 1994). Zn^{2+} seems to regulate PDE activities, because the same authors found that binding of cGMP to cGMP-specific PDE (type V) is activated by zinc concentrations up to 1 μM whereas zinc concentrations above 1 μM had inhibitory effects. Also for PDE IV A (Percival *et al.* 1997) and for PDE VI (He *et al.* 2000), a dependence of catalytic activity on zinc and an inhibition at higher zinc concentrations was shown, but these results cannot be generalized for all types of PDE. The cGMP-inhibited PDE (Omburo *et al.* 1995) and the cAMP-specific PDE (Kovala *et al.* 1997) are inhibited by zinc, but no activation at low zinc concentrations could be detected. In the latter case the authors discussed the possibility that zinc remained bound to the enzyme during the isolation process, so that zinc binding would not have been necessary for its activation. In conclusion, low concentrations of zinc seem to be essential for PDE activity while high concentrations have inhibitory effects, indicating a regulatory function of zinc for cyclic nucleotide phosphodiesterases.

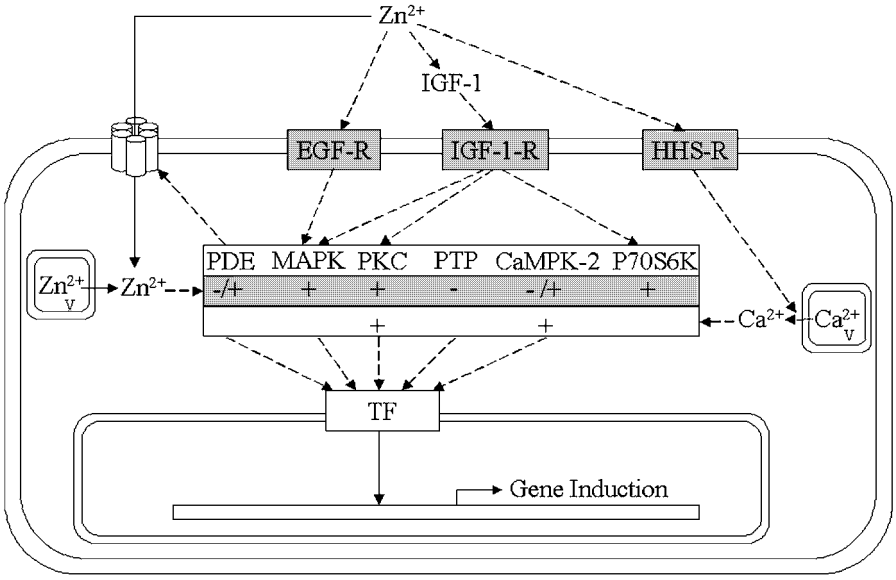

Figure 2. Effects of zinc on signal transduction pathways. Extracellular zinc can increase the formation of insulin-like growth factor (IGF), and stimulate the epidermal growth factor-receptor (EGR-R). The activation of a hepatic heavy metal ion stimulated-receptor (HHS-R) causes the intracellular release of Ca^{2+} in hepatocytes. At the level of protein phosphorylation, Zn^{2+} taken up and/or Zn^{2+} released from zincosomes can modulate the activity of cyclic nucleotide phosphodiesterase (PDE), mitogen-activated protein kinase (MAPK), protein kinase C (PKC), protein tyrosine phosphatases (PTP), Ca^{2+}-calmodulin activated protein kinase-2 (CaMPK-2), and P70S6 kinase (P70S6K). Activation of protein kinases or phosphatases leads to changes in the phosphorylation state of transcription factors (TF) and gene activities. Activating and inhibitory interactions are represented by + and −, respectively. Index V: vesicular localization.

In investigations with intact cells, these effects of zinc on the cGMP content were confirmed. Incubation of PC12 rat pheochromocytoma cells with zinc led to an increase of the cellular cGMP concentration, and in the homogenate of these cells a zinc-mediated inhibition of cGMP hydrolysis was identified as the cause for this effect (Waetjen *et al.* 2001). Recently, a new link between cGMP and zinc homeostasis was established. We observed, that not only zinc modulates cGMP signaling, but that cGMP also modulates the uptake of zinc (Haase 2001). In C6 rat glioma cells, zinc uptake was saturated after 1 h of incubation with 150 μM $ZnCl_2$. This type of saturation was not observed in the presence of LY 83583 or methylene blue, specific inhibitors of guanylate cyclase, and its appearance was accelerated by nitric oxide, an activator of soluble guanylate cyclase (Haase 2001). These observations have led us to the following hypothesis (Figure 3): Elevation of cellular Zn^{2+} results in a rise in cGMP, which inhibits further zinc import. This effect is only effective under conditions of ongoing cGMP synthesis and is abolished if guanylate cyclase is inhibited. At present we investigate whether the impairment of zinc import is caused by direct interaction of cGMP with an ion transporter or mediated by a cGMP-dependent protein kinase.

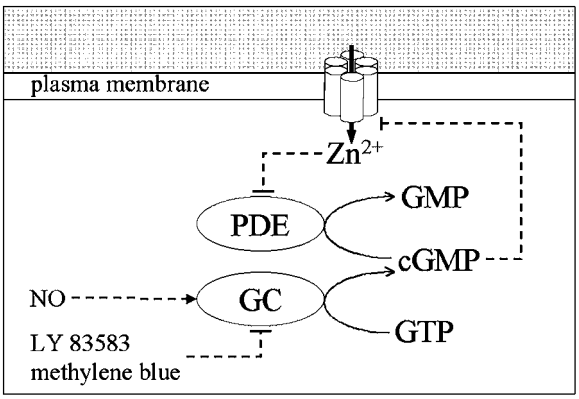

Figure 3. Mutual effects of zinc and cGMP-signaling. Increased intracellular zinc concentrations inhibit cyclic nucleotide phosphodiesterase (PDE), the cGMP-degrading enzyme. The resulting increase in cellular cGMP leads to a downregulation of zinc uptake, but only if the guanylate cyclase (GC) is not inhibited (e.g., by LY 83583 or methylene blue) and cGMP formation still can take place. Nitric monoxide (NO), an activator of GC, augments the downregulation of zinc uptake.

Receptor tyrosine kinases and mitogen-activated protein kinases

Several laboratories have investigated effects of zinc deficiency, chelation and/or zinc supplementation on mitogenic signaling pathways evoked by growth factors. Zinc deficiency specifically caused diminished levels of insulin-like growth factor-1 (IGF-1) both in humans (Cossack 1991) and rats (Dorup *et al.* 1991). In rat liver, zinc deficiency led to a decreased expression of growth hormone and IGF-1 genes (McNall *et al.* 1995). There is some controversy whether zinc stimulates the IGF-1 receptor activity directly. With NIH3T3 murine fibroblasts transfected with the IGF-1 receptor promoter, the zinc chelator DTPA did not affect phosphorylation of the IGF-1 receptor and of insulin receptor substrates (MacDonald 2000). However, zinc increased the affinity of both IGF-1 and IGF-2 to the type 1 IGF receptor on murine myoblasts with a half-maximal concentration of about 50 μM (McCusker *et al.* 1998). In accordance with this effect, zinc chelation by DTPA partially abolished the stimulation of mitogen-activated protein kinases (MAPK) by IGF-1 in rat fibroblasts, and this inhibition was reversed by addition of equimolar concentrations of zinc sulfate (Lefebvre *et al.* 1999). Conversely, elevation of extracellular zinc stimulated protein tyrosine phosphorylation and mitogen-activated protein kinase activity in murine fibroblasts (Hansson 1996). In human bronchial epithelial cells, various toxic metals and zinc induced EGF receptor phosphorylation and MAP kinase activation (Wu *et al.* 1999). The stimulating effect of zinc on tyrosine phosphorylation may be caused at least partially by interference with tyrosine dephosphorylation, because zinc inhibited various protein tyrosine phosphatases in human airway epithelial cells (Samet *et al.* 1999). This interpretation is in accordance with the observation that isolated recombinant protein tyrosine phosphatase was inhibited by zinc with an IC_{50} of 200 nM (Maret *et al.* 1999).

Incubation of mouse cortical cells with toxic zinc concentrations caused an activation of the MAP kinase ERK, leading to increased expression of the immediate early gene egr-1 (Park & Koh 1999). Treatment of human bronchial epithelial cells with subtoxic concentrations of Zn^{2+} activated the MAP kinases ERK, JNK and p38, and evoked increased phosphorylation of the transcription factors Jun and ATF-2, which are substrates of MAP kinases (Samet *et al.* 1998). A synergistic effect of zinc and calcium on DNA synthesis and mitogenic signaling was detected with NIH3T3 murine fibroblasts. When these cells were cultured in a medium containing 1.8 mM Ca^{2+} and starved from serum, supplementation with additional 1-2 mM Ca^{2+} and 40 μM Zn^{2+} markedly stimulated DNA synthesis and prolonged activation of MAPKs and P70S6 kinase (Huang *et al.* 1999). In Swiss 3T3 cells, phosphorylation and activation of P70S6 kinase was observed after treatment with zinc, and experiments with kinase inhibitors indicated activation through the phosphatidylinositol 3-kinase (PI3K) signaling pathway (Kim *et al.* 2000). Since PI3K is regulated by zinc proteins containing Zn^{2+} bound to a so-called FYVE domain (Wurmser *et al.* 1999), it may be speculated that this enzyme might be activated through Zn^{2+} binding to these PI3K regulating proteins. However, it is very difficult to predict effects of zinc on this system *in vivo*, because in the absence of Mg^{2+} micromolar concentrations of several transition metal ions including Zn^{2+} inhibited P70S6 kinase (Ferrari *et al.* 1991).

Protein kinase C structure and activity

The amino acid sequences of most isoforms of PKC contain two identical zinc-binding motifs at the N-terminus, the regulatory region of this enzyme (Parker *et al.* 1986). By site-directed mutagenesis it was demonstrated that at least one intact sequence is necessary for the binding of phorbol esters (Ono *et al.* 1989). Also other isoforms of PKC (α, βII, γ) were shown to contain four zinc atoms (Quest *et al.* 1992). EXAFS spectra of PKCβI indicated a complexation of each of the four zinc atoms by three cysteines and one histidine residue (Hubbard *et al.* 1991), and NMR spectroscopy of PKCα demonstrated that two non-consecutive sets of zinc-binding residues form two separate metal-binding sites (Hommel *et al.* 1994). A regulatory function of zinc for PKC is inferred from the observation that nanomolar concentrations of zinc can activate PKC and cause a translocation to the plasma membrane, a central event in the activation of PKC (Csermely *et al.* 1988a, b). Zinc also seems to regulate the translocation of PKC to the cytoskeleton (Zalewski *et al.* 1990). Furthermore, it was shown that a chelatable pool of intracellular zinc increases the binding of the PKC activator phorbol dibutyrate (Forbes *et al.* 1990) and that the zinc-binding cysteines are essential for phorbol ester binding (Ono *et al.* 1989).

It has been suggested that the above-mentioned four zinc-binding sites mediate the regulatory effects

of zinc on PKC. But on the other hand, the zinc finger-bound zinc could not even be removed by high affinity heavy metal ion chelators, making regulation by free zinc at these sites unlikely (Hubbard *et al.* 1991). Nevertheless, the chelators TPEN (Csermely *et al.* 1988b) and 1,10-phenanatroline (Forbes *et al.* 1990) were able to inhibit PKC activation. So the activation of PKC by zinc is mediated by a chelatable pool of zinc that is not identical with the metal ions bound to the zinc finger structures.

Furthermore, zinc was found to modulate the autonomous activity of PKC, i.e., the activity in the absence of activating cofactors. The oxidation of the zinc-binding cysteine residues led to a release of zinc and to an increase of the autonomous PKC activity, but to a loss of sensitivity to regulating cofactors (Knapp & Klann 2000). This indicates a possible involvement of the cellular redox state in PKC signaling mediated by the zinc finger structures.

Transcription factors

From gene sequencing data it is estimated that zinc is a structural element of more than a thousand transcription factors containing zinc finger domains (Berg & Shi 1996). With respect to regulatory functions of zinc, it is of special interest whether Zn^{2+} ions actually activate transcription factors to adopt specific promoter binding conformations. The best-characterized zinc-activated transcription factor is the metal response element-binding transcription factor-1 (MTF-1), which induces the metallothionein promoter in response to cellular zinc. MTF-1 contains six finger structures of which the first binds zinc with low affinity (Bittel *et al.* 1998). After complexing zinc, MTF-1 is translocated from the cytoplasm to the nucleus (Smirnova *et al.* 2000) where it binds to metal-response elements of MT promoters and the promoter from the zinc transporter ZnT-1 (Langmade *et al.* 2000). The activation of transcription factors by zinc is not restricted to MTF-1. In thyroid nuclear extracts, the human thyroglobulin enhancer is induced by the transcription factor CREB in synergism with a further factor, the CREB associated factor (CAF). CAF binding to DNA is abolished by 0.5 mM 1,10-phenanthroline and thus seems to depend on zinc (Berg *et al.* 1997). Furthermore, the binding of the negative regulator QM to the transcription factor Jun requires 1 μM Zn^{2+} and thus appears to be a zinc-regulated process (Inada *et al.* 1997). Zinc may also inhibit transcription factor activities directly. The activation of nuclear factor-κB in bovine cerebral cells is suppressed if cellular zinc is elevated by application of the zinc ionophore pyrithione (Kim *et al.* 1999). Also, the binding of steroids to the murine glucocorticoid receptor is reversibly inhibited by Zn^{2+} (Telford & Fraker 1997).

Zinc in cell proliferation, differentiation and apoptosis

Proven and putative regulatory functions of zinc in cell proliferation

Biochemical mechanisms for the function of zinc in cell proliferation were detected when zinc was shown to be a structural element in enzymes involved in DNA synthesis (Springgate *et al.* 1973; Chesters *et al.* 1989), transcription (Wu *et al.* 1992), aminoacyl-tRNA synthesis (Hicks & Wallwork 1987) and ribosomal function (Hard *et al.* 2000). Furthermore, zinc is present in the zinc finger structures of transcription factors that control the activity of genes responding to growth factors (Berg & Shi 1996). Zinc is not only a structural element but is also involved in regulatory mechanisms of cell proliferation. Based on observations that serum addition to mammalian cell cultures enhanced the cellular uptake of zinc, and that zinc deprivation by metal chelators caused decreased growth and DNA synthesis, zinc was proposed to be a second messenger of mitogenic signaling (Grummt *et al.* 1986). Taking the above aspects together, zinc was even suggested to act as a master hormone of growth and proliferation (Frausto da Silva & Williams 1991). Although this hypothesis cannot be substantiated in this general manner, there are several lines of evidence showing the central role of this element in the control of growth and proliferation. Zinc and calcium synergistically stimulated DNA synthesis and mitogenic signaling in murine fibroblasts (Huang *et al.* 1999). And conversely, treatment of Swiss 3T3 cells with the zinc chelator DTPA inhibited thymidine incorporation into DNA (Chesters *et al.* 1989) and impaired the transcription of the thymidine kinase gene (Chesters *et al.* 1990). The latter effect could be ascribed to the increased binding of an inhibitory transcription factor to the promoter of the thymidine kinase gene (Chesters *et al.* 1995).

Both zinc deficiency and zinc chelation caused impaired availability of growth hormones. In experiments with rats, zinc deficiency led to decreased secretion of growth hormone from the pituitary gland (Roth

& Kirchgessner 1994). As discussed above in the section on receptor tyrosine kinases, zinc deficiency specifically caused diminished levels of insulin-like growth factor-1 (IGF-1) both in humans and rats, and zinc deficiency led to a decreased expression of growth hormone and IGF-1 genes in the liver. The effects of zinc deficiency on the metabolism of IGF-1 are of special relevance, because this factor mediates the transition from G1- to S-phase of the cell cycle in cultured cells.

Several laboratories have investigated effects of zinc chelation and/or zinc supplementation on mitogenic signaling pathways evoked by growth factors (see section on signaling). Zinc stimulated protein tyrosine phosphorylation in murine fibroblasts and phosphorylation of the epidermal growth factor in human lung epithelial cells; it also stimulated various MAPK activities in murine fibroblasts and human lung epithelial cells, whereas zinc chelation interfered with the stimulation of MAP kinases by IGF-1 in rat fibroblasts. Treatment of human lung epithelial cells with Zn^{2+} activated the phosphorylation of the transcription factors Jun and ATF-2 (Wu *et al.* 1999). The enhancement of mitogen-activated kinase activities may be at least partially caused by the inhibition of protein phosphatases by Zn^{2+} (Maret *et al.* 1999, Samet *et al.* 1999).

A role for metallothionein in zinc-mediated cell proliferation

The cellular homeostasis of zinc is at least partially controlled by metallothionein, which has been shown to play a role in the regulation of cell proliferation. MT is overexpressed in proliferating tissues, e.g. in regenerating rat liver (Tsujikawa *et al.* 1994), in developing rat liver (Andrews *et al.* 1987) and in various tumors (Panemangalore *et al.* 1983; Cai *et al.* 1998). In cultured human colonic cancer cells (HT-29) cellular MT levels oscillated with the cell cycle and reached a maximum in successive G1-phases and at the G1- to S-transitions (Nagel & Vallee 1995). Hence, the cellular MT level could be an instrument of proliferation control. Not only the total cellular level but also the subcellular location of this protein is remarkably variable in the course of the cell cycle. Whereas MT is normally found in the cytoplasm and not in the nucleus, it accumulated transiently in the nuclear fraction of fetal and newborn rat livers, followed by redistribution to the cytoplasma 2–3 weeks post partum (Templeton *et al.* 1985; Nartey *et al.* 1987a). A translocation

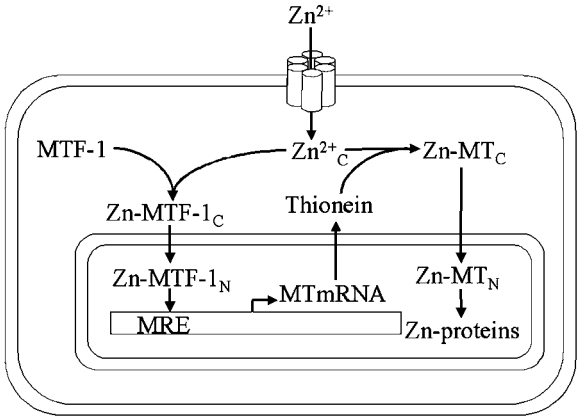

Figure 4. Model for interrelations between zinc and metallothionein. Binding of Zn^{2+} to the metal response element-binding transcription factor-1 (MTF-1) causes translocation of Zn-MTF-1_C into the nucleus. There, Zn-MTF-1_N binds a metal response element (MRE) in the promoter of the metallothionein (MT) gene. This interaction results in increased transcription and synthesis of thionein, which binds intracellular available Zn^{2+}. Index C: cytoplasmic localization; Index N: nuclear localization.

of MT into the nucleus was also observed during the early S-phase of growth factor-stimulated primary rat hepatocytes (Tsujikawa *et al.* 1991), in regenerating rat liver (Tsujikawa *et al.* 1994), certain tumors (Nartey *et al.* 1987b; Kuo *et al.* 1994; Woo & Lazo 1997) and in rat keratinocytes after irradiation with UV-B (Hanada *et al.* 1998). The nuclear translocation of MT probably is a vehicle for the achievement of a high nuclear zinc level in the S-phase of the cell cycle. Synchronized 3T3L1 fibroblasts show a nuclear accumulation of MT during the transition from G1- to S-phase of the cell cycle. When DNA synthesis was inhibited by aphidicolin, the cells were blocked at the G1- to S-transition, and zinc as well as MT were retained in the nucleus (Apostolova & Cherian 2000). After removal of aphidicolin, the cells reentered the S-phase, and both zinc and MT were relocated to the cytoplasm. The role of MT in these transitions seems to be to provide the increased amount of nuclear zinc required for DNA synthesis and mitogenic gene induction. The interrelations between zinc and MT during the onset of the S-phase of the cell cycle are summarized in Figure 4.

Cell differentiation

The overexpression of MT in developing tissues and at the transition from fetal to newborn rat development suggests a role for MT in differentiation, too. The differentiation of myoblasts to myotubes was inhibited by

the lack of zinc (Petrie *et al.* 1991). A novel role for zinc mediated by MT was found in the process of differentiation of 3T3L1 preadipocytes (Schmidt & Beyersmann 1999). After stimulation of differentiation by insulin and dexamethasone, these cells enter into a phase of rapid proliferation with a concomitant rise in cellular zinc and MT contents. Simultaneously MT is translocated from the cytoplasm into the nucleus. Upon entry of the cells into the subsequent actual differentiation, the elevated levels of zinc and MT return to the initial amounts, and a redistribution of MT to the cytoplasm occurs. Similar changes in subcellular localization of zinc and MT were also observed in the course of differentiation of two myoblast cell lines to myotubes (Apostolova *et al.* 1999). Induction of differentiation by lowering serum or addition of IGF-1 caused nuclear translocation of zinc and MT during early differentiation, whereas in fully differentiated myoblasts, MT was relocated to the cytoplasm and the total cellular MT content declined. At least three different protein kinases are involved in the nuclear translocation of MT in this system. Inhibitors of MAP-kinase kinase (PD98059), PI3-kinase (LY294092) and P70S6-kinase (rapamycin) all retained MT in the cytoplasm after induction of differentiation (Apostolova *et al.* 2000). Because MT itself is not phosphorylated by protein kinases, it has to be assumed that phosphorylation of a mediator protein not yet identified is required for nuclear translocation of MT. From these experiments it may be concluded that a high level of zinc is required for nuclear functions during the early stage of differentiation of some cell systems. It is tempting but premature to generalize that these roles of zinc and MT are not limited to the control of differentiation of preadipocytes and myoblasts, but occur in other systems, too.

Apoptosis

Generally, zinc protects from apoptosis induced by various agents. On the other hand, concentrations of extracellular zinc which exceed the capacity of homeostatic control may also induce programmed cell death in several mammalian cell lines. The mechanisms of induction of apoptosis by zinc are not well understood yet. For a discussion of the role of zinc in apoptosis, see Truong-Tran *et al.* (this issue).

Perspectives

Zn^{2+} ions should be regarded as possible cellular signaling factors. In some aspects, they exhibit features similar to those of regulatory free Ca^{2+} ions. As with Ca^{2+} there is a relatively strict control of the intracellular concentration of labile zinc available for binding to signaling proteins. Also similar to Ca^{2+}, there is a mechanism for sequestration of excess zinc in cytoplasmic vesicles and a control of nuclear translocation of the metal ion. In other aspects, zinc is different from calcium as a second messenger. Whereas the hormone-induced increases in free Ca^{2+} are short-lived transients of a few seconds, the changes in labile cellular zinc concentrations are much slower and longer lasting than those of Ca^{2+}. Furthermore, whereas for Ca^{2+} there exists the specific sensing protein calmodulin, which transmits the message from Ca^{2+} to the corresponding protein kinase, there exists no such general zinc sensor, but a host of zinc-dependent enzymes and transcription factors linked to DNA synthesis and gene expression. Zinc binding to and release from metallothionein is a tool to control the availability of zinc and its nucleocytoplasmic localization, but MT is not a signal transducing molecule in the strict sense as calmodulin is for Ca^{2+}.

There are many aspects of zinc functions that need further elucidation. E.g., we know too little about the physiology and biochemistry of zinc storage in vesicles and zinc translocation to and from the nucleus. Which signals regulate the total cellular zinc level and its intracellular distribution and the changes that can be observed during proliferation or differentiation? The level of intranuclear loosely bound zinc seems to be very low compared to the cytosolic level. The maintenance of this gradient requires an active and energy consuming but yet unidentified mechanism for the control of nuclear zinc, but we still lack knowledge about the nuclear functions of zinc. Possibly the nuclear availability of zinc is a mechanism for a control of gene expression, and MT may serve as suppliers for a controlled transfer of zinc to nuclear proteins.

If zinc serves as a signaling factor in the regulation of cell proliferation, differentiation and death, it is crucial to understand how specificity is achieved. Again, a comparison with the far better established regulatory mechanisms of calcium might bear the answer. Above all, the tissue-specific equipment of cells with receptors and signal processing intracellular proteins provides specificity. Within cells, zinc-mediated signals may be discriminated by compartmentation.

The cellular zinc storing vesicles, the so-called zincosomes, and the nucleocytoplasmic distribution of zinc are potential tools to secure target-specificity of zinc signals. At present, we know much less about the mechanisms of signaling by zinc than by calcium, and there is a need for future research in the field of specific zinc distribution and binding to functional targets.

References

Andrews GK, Gallant KR, Cherian MG. 1987 Regulation of the ontogeny of rat liver metallothionein mRNA by zinc. *Eur J Biochem* **166**, 527–531.

Apostolova MD, Ivanova IA, Cherian MG. 1999 Metallothionein and apoptosis during differentiation of myoblasts to myotubes: protection against free radical toxicity. *Toxicol Appl Pharmacol* **159**, 175–184.

Apostolova MD, Cherian MG. 2000 Delay of M-phase onset by aphidicolin can retain the nuclear localization of zinc and metallothionein in 3T3L1 fibroblasts. *J Cell Physiol* **183**, 247–253.

Apostolova MD, Ivanova IA, Cherian MG. 2000 Signal transduction pathways, and nuclear translocation of zinc and metallothionein during differentiation of myoblasts. *Biochem Cell Biol* **78**, 27–37.

Atar D, Backx PH, Appel MM, Gao WD, Marban E. 1995 Excitation-transcription coupling mediated by zinc influx through voltage-dependent calcium channels. *J Biol Chem* **270**, 2473–2477.

Benters J, Flögel U, Schäfer T, Leibfritz D, Hechtenberg S, Beyersmann D. 1997 Study of the interactions of cadmium and zinc ions with cellular calcium homeostasis using ^{19}F-NMR spectroscopy. *Biochem J* **322**, 793–799.

Berg JM, Shi Y. 1996 The galvanization of biology: A growing appreciation for the roles of zinc. *Science* **271**, 1081–1085.

Berg V, Vassart G, Christophe D. 1997 A zinc-dependent DNA-binding activity co-operates with cAMP-responsive-element-binding protein to activate the human thyroglobulin enhancer. *Biochem J* **323**, 349–357.

Bittel D, Dalton T, Samson SL, Gedamu L, Andrews GK. 1998 The DNA binding activity of metal response element-binding transcription factor-1 is activated in vivo and in vitro by zinc, but not by other transition metals. *J Biol Chem* **273**, 7127–7133.

Cai L, Wang GJ, Xu ZL, Deng DX, Chakrabarti S, Cherian MG. 1998 Metallothionein and apoptosis in primary human hepatocellular carcinoma (HCC) from northern China. *Anticancer Res* **18**, 4667–4672.

Cano-Gauci DF, Sarkar B. 1996 Reversible zinc exchange between metallothionein and the estrogen receptor zinc finger. *FEBS Lett* **386**, 1–4.

Canzoniero LMT, Sensi SL, Choi DW. 1997 Measurement of intracellular free zinc in living neurons. *Neurobiol Dis* **4**, 275–279.

Chesters JK, Petrie L, Vint H. 1989 Specificity and timing of the Zn^{2+} requirement for DNA synthesis by 3T3 cells. *Exp Cell Res* **184**, 499–508.

Chesters JK, Petrie L, Travis AJ. 1990 A requirement for Zn^{2+} for the induction of thymidine kinase but not ornithine decarboxylase in 3T3 cells stimulated from quiescence. *Biochem J* **272**, 525–527.

Chesters JK, Boyne R, Petrie L, Lipson KE. 1995 Role of the promoter in the sensitivity of human tymidine kinase to lack of Zn^{2+}. *Biochem J* **308**, 659–664.

Cossack ZT. 1991 Decline in somatomedin-C, insulin-like growth factor-1 with experimentally induced zinc deficiency in human subjects. *Clin Nutr* (Edinb.) **10**, 284–291.

Coyle P, Zalewski PD, Philcox JC, Forbes IJ, Ward AD, Lincoln SF, Mahadevan I, Rofe AM. 1994 Measurement of zinc in hepatocytes by using a fluorescent probe, Zinquin: Relationship to metallothionein and intracellular zinc. *Biochem J* **303**, 781–786.

Csermely P, Szamel M, Resch K, Somogyi J. 1988a Zinc increases the affinity of phorbol ester receptor in T lymphocytes. *Biochem Biophys Res Comm* **154**, 578–583.

Csermely P, Szamel M, Resch K, Somogyi J. 1988b Zinc can increase the activity of protein kinase C and contributes to its binding to the plasma membrane in T lymphocytes. *J Biol Chem* **263**, 6487–6490.

Cuajungco MP, Lees GJ. 1997. Zinc metabolism in the brain: relevance to human neurodegenerative disorders. *Neurobiol Dis* **4**, 137–169.

Donnelly TE. 1978 Effects of zinc chloride on the hydrolysis of cyclic GMP and cyclic AMP by the activator-dependent cyclic nucleotide phosphodiesterase from bovine heart. *Biochim Biophys Acta* **522**, 151–160.

Dorup I, Flyvbjerg A, Everts ME, Clausen T. 1991 Role of insulin-like growth factor-1 and growth hormone in growth inhibition induced by magnesium and zinc deficiencies. *Br J Nutr* **66**, 505–521.

Ferrari S, Bandi HR, Hofsteenge J, Bussian BM, Thomas G. 1991 Mitogen-activated 70K S6 kinase. Identification of in vitro 40 S ribosomal S6 phosphorylation sites. *J Biol Chem* **266**, 22770–22775.

Forbes IJ, Zalewski PD, Giannakis C, Petkoff HS, Cowled PA. 1990 Interaction between protein kinase C and regulatory ligand is enhanced by a chelatable pool of cellular zinc. *Biochim Biophys Acta* **1053**, 113–117.

Francis SH, Colbran JL, McAllister-Lucas LM, Corbin JD. 1994 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.

Frausto da Silva JJR, Williams RJP. 1991 The biological chemistry of the elements. *Clarendon Press, Oxford,* 299–318.

Grummt F, Weinmann-Dorsch C, Schneider-Schaulies J, Lux A. 1986 Zinc as a messenger of mitogenic induction. Effects on diadenosine tetraphosphate (Ap_4A) and DNA synthesis. *Exp Cell Res* **163**, 191–200.

Haase H. 2001 Zinkhomöostase in Säugerzellen: Untersuchungen zur Aufnahme, intrazellulären Verteilung und Toxizität. *GCA-Verlag, Herdecke*, ISBN 3-89863-024-2.

Haase H, Beyersmann D. 1999 Uptake and intracellular distribution of labile and total Zn(II) in C6 rat glioma cells investigated with fluorescent probes and atomic absorption. *Biometals* **12**, 247–254.

Hanada K, Tamai K, Sawamura D, Hashimoto I, Muramatsu T. 1998 Dynamic changes in intracellular location of metallothionein in rat keratinocytes after ultraviolet-B irradiation. *J Invest Dermatol* **110**, 98–100.

Hansson A. 1996 Extracellular zinc ions induce mitogen-activated protein kinase activity and protein tyrosine phosphorylation in bombesin-sensitive Swiss 3T3 fibroblasts. *Arch Biochem Biophys* **328**, 233–238.

Hard T, Rak A, Allard P, Kloo L, Garber M. 2000 The solution structure of ribosomal protein L36 from Thermus thermophilus reveals a zinc-ribbon-like fold. *J Mol Biol* **296**, 169–180.

He F, Seryshev AB, Cowan CW, Wensel TG. 2000 Multiple zinc binding sites in retinal rod cGMP phosphodiesterase, PDE6$\alpha\beta$. *J Biol Chem* **275**, 20572–20577.

Hicks SE, Wallwork JC. 1987 Effect of dietary zinc deficiency on protein synthesis in cell-free systems isolated from rat liver. *J Nutr* **117,** 1234–1240.

Hommel U, Zurini M, Luyten M. 1994 Solution structure of a cysteine rich domain of rat protein kinase C. *Nat Struct Biol* 1, 383–387.

Huang JS, Mukherjee JJ, Chung T, Crilly KS, Kiss Z. 1999 Extracellular calcium stimulates DNA synthesis in synergism with zinc, insulin and insulin-like growth factor I in fibroblasts. *Eur J Biochem* **266**, 943–951.

Hubbard SR, Bishop WR, Kirschmeier P, George SJ, Cramer SP, Hendrickson WA. 1991 Identification and characterization of zinc binding sites in protein kinase C. *Science* **254**, 1776–1779.

Inada H, Mukai J, Matsushima S, Tanaka T. 1997 QM is a novel zinc-binding transcriptional regulatory protein: its binding to c-jun is regulated by zinc ions and phosphorylation by protein kinase C. *Biochem Biophys Res Commun* **230**, 331–334.

Jacob C, Maret W, Vallee BL. 1998 Control of zinc transfer between thionein, metallothionein, and zinc proteins. *Proc Natl Acad Sci USA* **95**, 3489–3494.

Kim CH, Kim JH, Moon SJ, Chung KC, Hsu CY, Seo JT, Ahn YS. 1999 Pyrithione, a zinc ionophore, inhibits NF-κB activation. *Biochem Biophys Res Commun* **259**, 505–509.

Kim S, Jung Y, Kim D, Koh H, Chung J. 2000 Extracellular zinc activates p70S6 kinase through the phosphatidylinositol 3-kinase signaling pathway. *J Biol Chem* **275**, 25979–25984.

Knapp LT, Klann E. 2000 Superoxide-induced stimulation of protein kinase C via thiol modification and modulation of zinc content. *J Biol Chem* **275**, 24136–24145.

Kovala T, Sanwal BD, Ball EH. 1997 Recombinant expression of a Type IV, cAMP-specific phosphodiesterase: characterization and structure-function studies of deletion mutants. *Biochemistry* **36**, 2968–2976.

Kuo SM, Kondo Y, DeFillipo JM, Einstorff MS, Bahuson RR, Lazo JS. 1994 Subcellular localization of metallothionein IIA in human bladder tumor cells using a novel epitope-specific antiserum. *Toxic Appl Pharmacol* **125**, 104–110.

Langmade SJ, Ravindra E, Daniels PJ, Andrews GK. 2000 The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. *J Biol Chem* **275**, 34803–34809.

Lefebvre D, Boney CM, Ketelslegers JM, Thissen JP. 1999 Inhibition of insulin-like growth factor-I mitogenic action by zinc chelation is associated with a decreased mitogen-activated protein kinase activation in RAT 1 fibroblasts. *FEBS Lett* **449**, 284–288.

Lengyel I, Fieuw-Makaroff S, Hall AL, Sim ATR, Rostas JAP, Dunkley PR. 2000 Modulation of the phosphorylation and activity of calcium/calmodulin-dependent protein kinase II by zinc. *J Neurochem* **75**, 594–605.

MacDonald RS. 2000 The role of zinc in growth and cell proliferation. *J Nutr* **130**, 1500S–1508S.

Maret W, Larsen KS, Vallee BL. 1997 Coordination dynamics of biological zinc 'clusters' in metallothioneins and in the DNA binding domain of the transcription factor Gal4. *Proc Natl Acad Sci USA* **94**, 2233–2237.

Maret W, Jacob C, Vallee BL, Fischer EH. 1999 Inhibitory sites in enzymes: Zinc removal and reactivation by thionein. *Proc Natl Acad Sci USA* **96**, 1936–1940.

McCusker RH, Kaleko M, Sackett RL 1998 Multivalent cations and ligand affinity of the type 1 insulin-like growth factor receptor on P2A2-LISN muscle cells. *J Cell Physiol* **176**, 392–401.

McNall AD, Etherton TD, Fosmire GJ. 1995 The impaired growth induced by zinc deficiency in rats is associated with decreased expression of the hepatic insulin-like growth factor I and growth hormone receptor genes. *J Nutr* **125**, 874–879.

McNulty TJ, Taylor CW. 1999 Extracellular heavy-metal ions stimulate Ca^{2+} mobilization in hepatocytes. *Biochem J* **339**, 555–561.

Nagel WW, Vallee BL. 1995 Cell cycle regulation of metallothionein in human colon cancer cells. *Proc Natl Acad Sci USA* **92**, 579–583.

Nartey NO, Banerjee D, Cherian MG. 1987a Immunohistochemical localization of metallothionein in cell nucleus and cytoplasm of fetal human liver and kidney and its changes during development. *Pathology* **119**, 233–238.

Nartey NO, Cherian MG, Banerjee D. 1987b Immunohistochemical localization of metallothionein in human thyroid tumors. *Am J Pathol* **129**, 177–182.

Omburo GA, Brickus T, Gazaleh FA, Colman RW. 1995 Divalent metal cation requirement and possible classification of cGMP-inhibited phosphodiesterase as a metallohydrolase. *Arch Biochem Biophys* **323**, 1–5.

Ono Y, Fujii T, Igarashi K, Kuno T, Tanaka C, Kikkawa U, Nishizuka Y. 1989 Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. *Proc Natl Acad Sci USA* **86**, 4868–4871.

Panemangalore M, Banerjee D, Okosaka S, Cherian MG. 1983 Changes in the intracellular accumulation and distribution of metallothionein in rat liver and kidney during postnatal development. *Dev Biol* **97**, 95–102.

Park JA, Koh JY. 1999 Induction of an immediate early gene egr-1 by zinc through extracellular signal-related kinase activation in cortical culture: its role in zinc-induced neuronal death. *J Neurochem* **73**, 450–456.

Parker P, Coussens L, Totty N, Rhee L, Young S, Chen E, Stabel S, Waterfield MD, Ullrich A. 1986 The complete primary structure of protein kinase C – the major phorbol ester receptor. *Science* **233**, 853–859.

Percival MD, Yeh B, Falgueyret JP. 1997 Zinc dependent activation of cAMP-specific phosphodiesterase (PDE4A). *Biochem Biophys Res Commun* **241**, 175–180.

Petrie L, Chesters JK, Franklin M. 1991 Inhibition of myoblast differentiation by lack of zinc. *Biochem J* **276**, 109–111.

Qian WJ, Aspinwall CA, Battiste MA, Kennedy RT. 2000 Detection of secretion from single pancreatic β-cells using extracellular fluorogenic reactions and confocal fluorescence microscopy. *Anal Chem* **72**, 711–717.

Quest AFG, Bloomenthal J, Bardes ESG, Bell RM. 1992 The regulatory domain of protein kinase C coordinates four atoms of zinc. *J Biol Chem* **267**, 10193–1197.

Roth HP, Kirchgessner M. 1994 Influence of alimentary zinc deficiency on the concentration of growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin in the serum of force-fed rats. *Horm Metab Res* **26**, 404–408.

Samet JM, Silbajoris R, Wu W, Graves LM. 1999 Tyrosine phosphatases as targets in metal-induced signaling in human airway epithelial cells. *Am J Resp Cell Mol Biol* **21**, 357–364.

Samet JM, Graves LM, Quay J, Dailey LA, Devlin RB, Ghio AJ, Wu W, Bromberg PA, Reed W. 1998 Activation of MAPKs in human bronchial epithelial cells exposed to metals. *Am J Physiol* **275**, L551–L558.

Schmidt C, Beyersmann D. 1999 Transient peaks in zinc and metallothionein levels during differentiation of 3T3L1 cells. *Arch Biochem Biophys* **364**, 91–98.

Sensi SL, Canzoniero LMT, Yu SP, Ying HS, Koh JY, Kerchner GA, Choi DW. 1997 Measurement of intracellular free zinc in living cortical neurons: Routes of entry. *J Neurosci* **17**, 9554–9564.

Simons TJ. 1991 Intracellular free zinc and zinc buffering in human red blood cells. *J Membr Biol* **123**, 63–71.

Smirnova IV, Bittel DC, Ravindra R, Jiang H, Andrews GK. 2000 Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. *J Biol Chem* **275**, 9377–9384.

Springgate CF, Mildvan AS, Abramson R, Engle JL, Loeb LA. 1973 Escherichia coli deoxyribonucleic acid polymerase I, a zinc metalloenzyme. Nuclear quadrupole relaxation studies of the role of bound zinc. *J Biol Chem* **248**, 5987–5993.

Telford WG, Fraker PJ. 1997 Zinc reversibly inhibits steroid binding to murine glucocorticoid receptor. *Biochem Biophys Res Commun* **238**, 86–89.

Templeton DM, Banerjee MD, Cherian MG. 1985 Metallothionein synthesis and localization in relation to metal storage in rat liver during gestation. *Can J Biochem Cell Biol* **63**, 16–22.

Tsujikawa K, Imai T, Kakutani M, Kayamori Y, Mimura T, Otak N, Kimura M, Fukuyama R, Shimizu N. 1991 Localization of metallothionein in nuclei of growing primary cultured adult rat hepatocytes. *FEBS Lett* **283**, 239–242.

Tsujikawa K, Suzuki N, Sagawa K, Itoh M, Sugiyama T, Kohama Y, Otaki N, Kimura M, Mimura T. 1994 Induction and subcellular localization of metallothionein in regenerating rat liver. *Eur J Cell Biol* **63**, 240–246.

Udom AO, Brady FO. 1980 Reactivation in vitro of zinc-requiring apo-enzymes by rat liver zinc-thionein. *Biochem J* **187**, 329–335.

Vallee BL, Falchuk KH. 1993 The biochemical basis of zinc physiology. *Physiol Rev* **73**, 79–118.

Waetjen W, Benters J, Haase H, Schwede F, Jastorff B, Beyersmann D. 2001 Zn^{2+} and Cd^{2+} increase the cyclic GMP level in PC 12 cells by inhibition of the cyclic nucleotide phosphodiesterase. *Toxicology* **157**, 167–175.

Walkup GK, Burdette SC, Lippard SJ, Tsien RY. 2000 A new cell-permeable fluorescent probe for Zn^{2+}. *J Am Chem Soc* **122**, 5644–5645.

Woo ES, Lazo JS. 1997 Nucleocytoplasmic functionality of metallothionein. *Cancer Res* **57**, 4236–4241.

Wu FY, Huang WJ, Sinclair RB, Powers L. 1992 The structure of the zinc sites of Escherichia coli DNA-dependent RNA polymerase. *J Biol Chem* **267**, 25560–25567.

Wu W, Graves LM, Jaspers I, Devlin RB, Reed W, Samet JM. 1999 Activation of the EGF receptor signaling pathway in human airway epithelial cells exposed to metals. *Am J Physiol* **277**, L924–L931.

Wurmser AE, Gary JD, Emr SD. 1999. Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways. *J Biol Chem* **274**, 9129–9132.

Zalewski PD, Forbes IJ, Betts WH. 1993 Correlation of apoptosis with change in intracellular labile Zn(II) using Zinquin [(2-methyl-8-*p*-toluenesulphonamido-6-quinolyloxy) acetic acid], a new specific fluorescent probe for Zn(II). *Biochem J* **296**, 403–408.

Zalewski PD, Forbes IJ, Giannakis C, Cowled PA, Betts WH. 1990 Synergy between zinc and phorbol ester in translocation of protein kinase C to cytoskeleton. *FEBS Lett* **273**, 131–134.

Zeng J, Heuchel R, Schaffner W, Kägi JHR. 1991 Thionein (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. *FEBS Lett* **279**, 310–312.