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*Critical Review*

# Zinc Deficiency in Neuronal Biology

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***Summary***

**Adverse nutritional and environmental conditions during early development can irreversibly affect the nervous system. Zinc (Zn) deficiency associated with inadequate Zn intake and undernutrition is frequent throughout the world. Increasing evidence indicates that developmental Zn deficiency can lead to alterations in neonate and infant behavior, cognitive and motor performance that persist into adulthood. This review will address current knowledge on the events that are triggered in neuronal cells when Zn availability decreases and discuss their consequences on neuronal function and development. In neuronal cells, Zn deficiency induces oxidative stress, alters the normal structure and dynamics of the cytoskeleton, affects the modulation of transcription factors AP-1, NF-κB and NFAT and induces a decreased cell proliferation and increased apoptotic death. Thus, these closely associated events can affect neuronal function and critical developmental events (neuronal proliferation, differentiation, plasticity and survival) when Zn availability decreases.**
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**Keywords** Zinc; zinc deficiency; apoptosis; NF-κB; AP-1; NFAT; reactive oxygen species; cytoskeleton; microtubules; nuclear transport.

**Abbreviations** CGN, cerebellum granule neurons; ERK, extracellular signal-regulated kinase; JNK, c-Jun-N-terminal kinase; $H_2O_2$, hydrogen peroxide; MAPKs, mitogen-activated protein kinases; NAC, N-acetylcysteine; Zn, zinc.

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## INTRODUCTION

Based on National food balance data (1992–2000) for different world regions, it was recently estimated that the percentage of individuals at risk of inadequate zinc (Zn) intake ranged between 9.5% (USA) and 33.1% (Southeast Asia) (*1*). Analysis of data from the third National Health and Nutrition Survey (NHANES III), 1988–1994, indicates that only 56% of the total USA population has adequate Zn intakes (*2*). Although differences in the approaches to evaluate adequate Zn intake can lead to important variations, it is clear that a significant percentage of the population worldwide can be at risk of marginal Zn intake.

Zn is a key micronutrient in the physiology of the nervous system (*3*). The developing brain can be highly sensitive to a deficit of this nutrient for several reasons, including the need of Zn for appropriate cell differentiation, migration, and growth. Developmental severe Zn deficiency is characterized by a high frequency of brain and eye malformations, including agenesis and dysmorphogenesis of the brain, spinal cord, eye, and olfactory tract (*4, 5*). Developmental and early postnatal marginal Zn deficiency affects the expression of Zn transporters (*6*) and of the NMDA receptor (NMDAR) subunits in rat brain (*7*).

Zn deprivation during critical developmental periods can result in altered emotionality and food motivation early in life (*8, 9*). Developmental Zn deficiency in humans is associated with impaired neonatal/infant behavior, cognitive and motor performance (*10–12*). Importantly, Zn supplementation of undernourished children improves their developmental quotients, activity patterns and neuropsychological functions (*10–13*). While the deleterious effects of gestational or perinatal Zn deficiency on brain development/function are well documented, the underlying mechanisms of these adverse effects remain largely unknown.

A significant body of research has focused on the Zn-containing neurons and on the participation of Zn in neurotransmission. Neuronal injury secondary to *in vivo* Zn

mobilization and release occurs in several traumatic conditions, such as prolonged seizures and transient cerebral ischemia, and in neurodegenerative processes (*3*). The neurobiology of Zn-enriched neurons is not the focus of this review, and has been recently reviewed by Frederickson et al. (*3*).

There is limited information on the functional consequences of a decreased Zn availability to neurons. Neurons are possible targets of the deleterious effects of Zn deficiency. Postnatal Zn deficiency in rats can result in an impaired development of the cerebellum (*14*), with persistence of the external granule cell layer, a thinner molecular layer and a decreased area of the internal granule cell layer (*15*). Zn deficiency imposed to rats during the first three postnatal weeks can affect the basket and stellate (*16*), Purkinje (*17*) and cerebellum granule neurons (CGN) (*15*). Zn deficiency caused an impaired dendritic differentiation in basket and stellate cells that may be secondary to a delayed onset of dendritic differentiation and dendritic growth rate (*16*). A delayed maturation of the Purkinje cells, with marked dendritic alterations, and a reduced number of CGN has also been observed in Zn deficient rats (*15, 17*). In support of the deleterious effects of Zn deficiency on neurons, we have observed that in human neuroblastoma IMR-32 cells, Zn deficiency is associated with: (a) an increased production of oxidants (*18*); (b) impaired tubulin polymerization (*19, 20*); (c) altered modulation of transcription factors AP-1 (*21*), NF-$\kappa$B (*19, 20*) and NFAT (*22*); and (d) increased apoptotic death (*23*).

This review will summarize current knowledge on the consequences of decreased Zn availability on different aspects of neuronal physiology. Alterations in neuronal proliferation, migration, differentiation and survival during critical developmental periods could be one underlying cause of the altered motor and cognitive functions associated with Zn deficiency.

## ZINC DEFICIENCY AND NEURONAL OXIDATIVE STRESS

Zn deficiency can lead to a condition of oxidative stress which has been consistently observed in cell and animal models (*24–27*). Zn deficiency is associated with higher than normal levels of tissue oxidative damage, including increased lipid, protein and DNA oxidation (*24, 25, 28*) and with altered activity and concentration of enzymes and other components of the oxidant defense system (*27, 29*).

In human neuroblastoma cells, the exposure to media containing low Zn concentrations leads to a rapid decrease in cellular labile Zn and total Zn concentrations. Labile Zn pools decreased within 3 h in the Zn deficient cells. After 24 h, Zn deficient cells showed a 35% decrease in labile Zn and 27% decrease in total Zn concentrations compared to controls (Fig. 1A) (*20*). The decrease in cellular Zn is accompanied by an increase in neuronal oxidant species. After 24 h in the Zn deficient media, neuronal cells show an increase in total cell oxidants that is prevented when the media is supplemented with 15 $\mu$M Zn (Fig. 1B and C). One of the main oxidant species that increases with Zn deficiency in IMR-32 cells is hydrogen peroxide ($H_2O_2$) (Fig. 1B). Simultaneously, several components of the oxidant defense system are affected by Zn deficiency. Glutathione, the main cellular non-protein thiol and a fundamental component of neuronal protection against oxidative stress, decreases in neuronal cells with Zn deficiency (*18*). Supplementation with $\alpha$-lipoic acid and N-acetylcysteine (NAC) can restore the low glutathione concentrations in Zn deficient cells, presumably as a consequence of their ability to prevent the increase in cellular $H_2O_2$ and the replenishment of reduced glutathione by NAC. Among the enzymatic components of the oxidant defense system, the activity of CuZn superoxide dismutase (Fig. 2A) and the activity and expression of Mn superoxide dismutase (Fig. 2A, B and C) are increased in Zn deficient neuroblastoma cells compared to Zn adequate controls. These enzymes catalyze the transformation of superoxide anion to $H_2O_2$. The activity of glutathione peroxidase, which metabolizes peroxides, and glutathione reductase, which reduces oxidized glutathione, were not affected by Zn deficiency (Fig. 2A).

The mechanisms underlying the increase in cellular oxidants associated with Zn deficiency are still poorly understood. Zn deficiency-induced oxidative stress may be related to the antioxidant properties of Zn, including its capacity to displace redox active metals (copper, iron) from binding sites in macromolecules (*30*) or to secondary alterations of other cellular events. The decreased expression of metallothionein associated with Zn deficiency could also increase the susceptibility to oxidative stress since metallothionein is a key protein in the regulation of both, thiol and Zn homeostasis (*31*). Furthermore, an altered mitochondrial function due to altered expression of components of the respiratory chain was proposed as a source of oxidants in Zn deficiency (*32*). However, the observed increase in neuronal oxidants can be detected within one hour of incubating neurons in Zn deficient media (data not shown). This suggests that initially, other mechanisms that do not require protein synthesis are involved in neuronal oxidant increase.

We have recently found that cellular calcium increases within 3 h of incubating IMR-32 neuroblastoma cells in Zn deficient media (Fig. 1A) (*33*). A rise in cytosolic calcium could activate nitric oxide synthase 1 via calmodulin-regulated events leading to high NO production (*34*). A rise in cytosolic calcium could also activate NADPH oxidase causing an increased production of reactive oxygen species (*35*). Although not all NADPH oxidase isoforms are activated by calcium, the activation of calcium-sensitive protein kinase C could cause NADPHox activation of calcium-insensitive isoforms. However, the feasibility of these mechanisms needs to be established.

## ZINC AND THE NEURONAL CYTOSKELETON

Alterations in tubulin polymerization have been described in association with Zn deficiency in fetal and adult rat brain and in human neuroblastoma cells (*20, 36, 37*). Hesketh (1981)

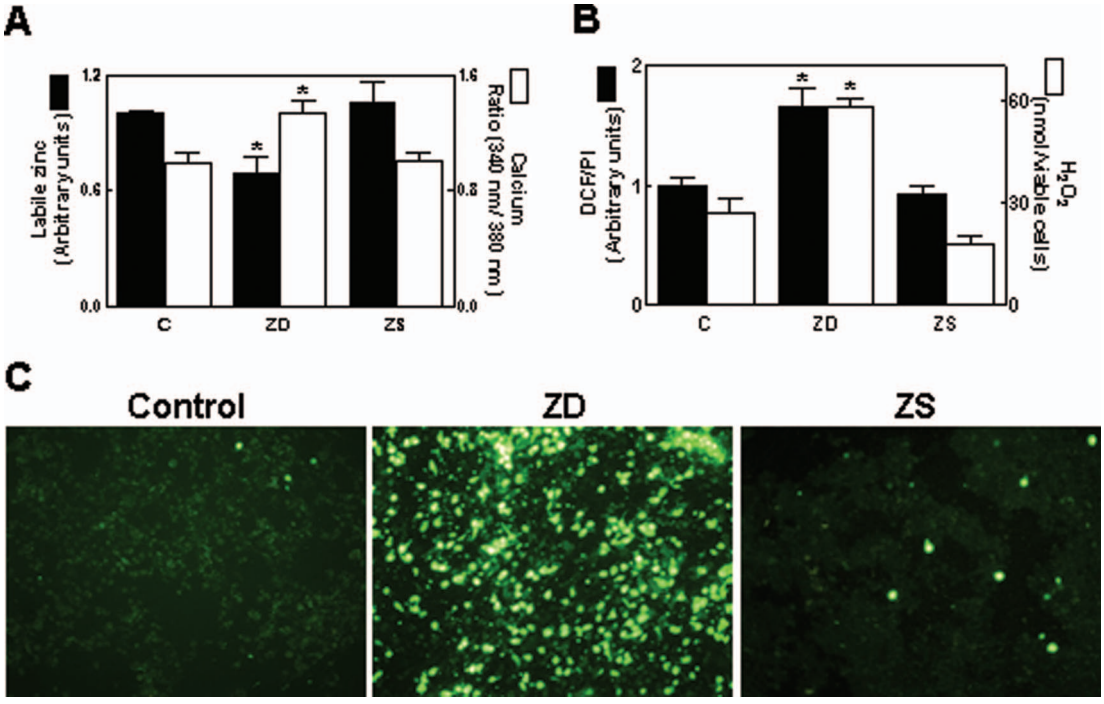


**Figure 1.** Zn deficiency induces an increase in cellular oxidant levels and $H_2O_2$ release in human neuroblastoma IMR-32 cells. (A) Labile zinc levels (full bars) were determined with the probe *N*-6-(6-Methoxy-8-quinolyl)-*p*-toluenesulfonamide (TSQ) as described in (*20*) and cellular calcium levels (empty bars) were determined with the probe Fura2-AM as described in (*22*), after 24 h of incubation in the corresponding media. Results are shown as means $\pm$ SEM of 4 independent experiments. *Significantly different compared to C and ZS groups ($P < 0.05$, one way ANOVA test). (B) Cellular oxidant levels (full bars) and $H_2O_2$ released to the media (empty bars) were measured following the experimental protocols described in (*18*). DCDHF fluorescence was normalized to the propidium iodide fluorescence. $H_2O_2$ levels were normalized to cell viability values. Results are shown as means $\pm$ SEM of 4 independent experiments. *Significantly different compared to all other tested conditions ($P < 0.01$, one way ANOVA test). (C) Cellular oxidant levels were measured in IMR-32 cells after 24 h of culturing cells in control (C), Zn deficient (ZD), or Zn supplemented (ZS) media. Global oxidant levels were determined with the probe 5(or 6)-carboxy-2′7′-dichlorodihydrofluorescein diacetate that enters the cells and fluoresces when oxidized. Fluorescence microscopy of cells incubated in control (left panel), ZD (central panel) or ZS (right panel) media. Fig. 1A is adapted from (*20, 22*). Fig. 1B is an adaptation of results from (*18*).

initially reported that tubulin reassembly was affected in brain supernatants from Zn deficient rats and pigs (*36*). Zn deficiency affects the kinetics of brain tubulin polymerization. A lower initial velocity and a longer lag period in *in vitro* tubulin assembly was observed in brain supernatants obtained from Zn deficient rats compared to Zn supplemented control (*20, 36, 37*). In human IMR-32 cells, Zn deficiency affects tubulin polymerization and the structure of the microtubule network. The rate of *in vitro* tubulin polymerization in supernatants from Zn deficient cells was lower than in control or in Zn supplemented cells (Fig. 3A and B). Although Zn deficiency does not affect the total content of tubulin (Fig. 3C), the amount of polymerized tubulin is lower in Zn deficient IMR-32 cells (Fig. 3B). Zinc deficiency also affects the structure of microfilaments in IMR-32 cells (*19*). The relationship between actin and tubulin disruption when neuronal Zn decreases needs to be established.

The association of a condition of Zn deficiency with a decrease in tubulin polymerization has been known for many years. However, the underlying mechanisms as well as the consequences of tubulin dynamics dysfunction on cell physiology are still unknown. Current evidence indicates that the cell cytoskeleton participates in the modulation of cell signaling through different mechanisms of action. In neurons, intracellular trafficking and localization of subcellular elements such as vesicles, organelles and proteins (including transcription factors) is particularly complex due to the long distance trafficking/signaling that occurs through the axons. In this complex dynamics, the long-distance transport is mediated by the microtubules, which mainly consist of motor proteins moving along polarized microtubule tracks. In the next section, we discuss the impact of Zn deficiency-induced microtubule disruption on cell signaling.

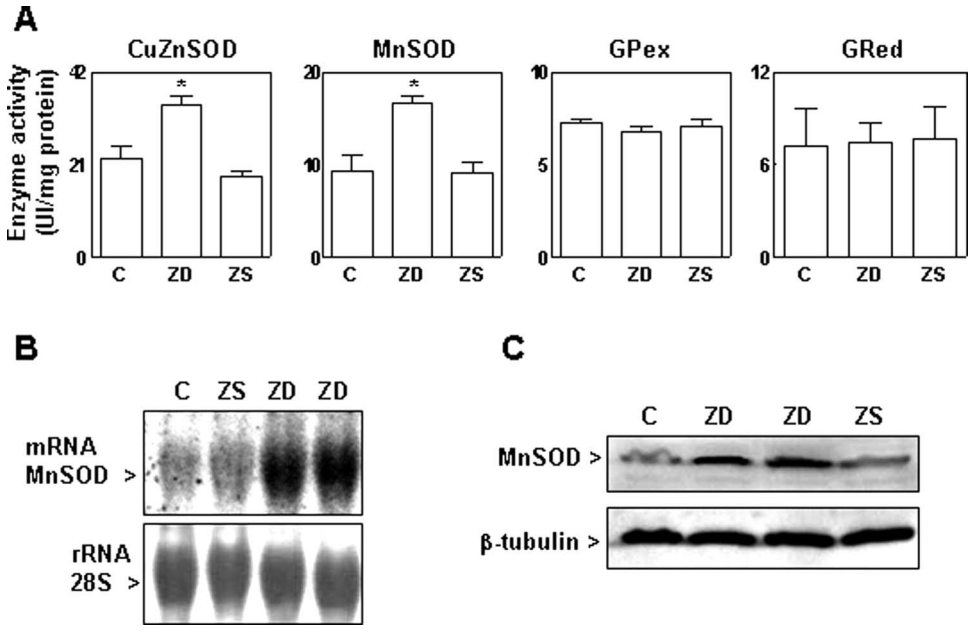


**Figure 2.** Zn deficiency selectively modulates antioxidant defense enzyme activities in human neuroblastoma IMR-32 cells. (A) CuZn superoxide dismutase (CuZnSOD), Mn superoxide dismutase (MnSOD), glutathione peroxidase (GPex) and glutathione reductase (GRed) activities were measured in $10{,}000 \times g$ supernatants from cells exposed to control (C), Zn deficient (ZD) or Zn supplemented (ZS) media for 24 h. Results are presented as means $\pm$ SEM and are the average of 4 independent experiments. *Significantly different from the C and ZS groups ($P < 0.05$) (one-way ANOVA test). (B) MnSOD mRNA levels were measured by Northern blot, and (C) MnSOD protein content was measured by Western blot after cells were exposed to control (C), Zn deficient (ZD) or Zn supplemented (ZS) media for 48 h. rRNA 28S and $\beta$-tubulin levels were used as loading controls. Representative Northern and Western blots out of 3 independent experiments are shown.

## ZINC DEFICIENCY AND NEURONAL SIGNALING

A decrease in neuronal Zn affects key cell signals involved in neuronal proliferation, differentiation and survival. Zn deficiency influences the activity of transcription factors AP-1, NF-$\kappa$B and NFAT, which are sensitive to oxidants, thiol redox status, and conditions of oxidative stress (*18, 21, 24*). In neuronal cells, Zn deficiency is in general associated with the activation of AP-1 and with alterations in NF-$\kappa$B- and NFAT-dependent gene transcription. Below, we summarize our present knowledge on AP-1, NF-$\kappa$B and NFAT modulation in neuronal cells when Zn availability decreases.

### *AP-1*

A broad range of physiological stimuli and stress conditions activate AP-1, leading to the modulation of important cellular processes including the decision of cells to proliferate, differentiate, survive, or die by apoptosis. Extensive evidence supports the involvement of $H_2O_2$ in the triggering of AP-1. In rat cortical neurons and astrocytes, $H_2O_2$ activates mitogen-activated protein kinases (MAPKs) (*38*), AP-1 and c-Jun expression, with a higher activation in neurons than in astrocytes (*39*). Upstream of AP-1, the stress-related MAPKs, c-Jun-N-terminal kinase (JNK) and p38, are also activated by increases in the intracellular levels of oxidants (*40*). MAPKs and AP-1 are implicated in normal physiological functions of the brain. c-Jun, a component of AP-1, has been recently attributed a bipotential role mediating neurodegeneration and cell death, as well as participating in plasticity and repair mechanisms (*41*).

Zn deficiency triggers the activation of the stress-responsive MAPKs, JNK and p38, and of the upstream MAPK kinase (SEK1/MKK4), leading to increased AP-1-DNA binding activity in nuclear and total cell fractions, and increased AP-1-dependent gene expression in IMR-32 cells (Fig. 4) (*21*). The activation of JNK, p38 and AP-1 was triggered by an increase in $H_2O_2$, since treatment of Zn deficient cells with catalase, an enzyme that metabolizes $H_2O_2$, prevented their occurrence (*21*). Although the functional consequences of AP-1 activation via JNK and p38 in Zn deficiency need to be established, we found that these events could be involved in the sensitization of neurons to metal toxicity. In this regard, a condition of Zn deficiency potentiates $Pb^{2+}$-induced increase in cell oxidants, activation of p38, JNK and AP-1, and decreased cell viability (*42*).

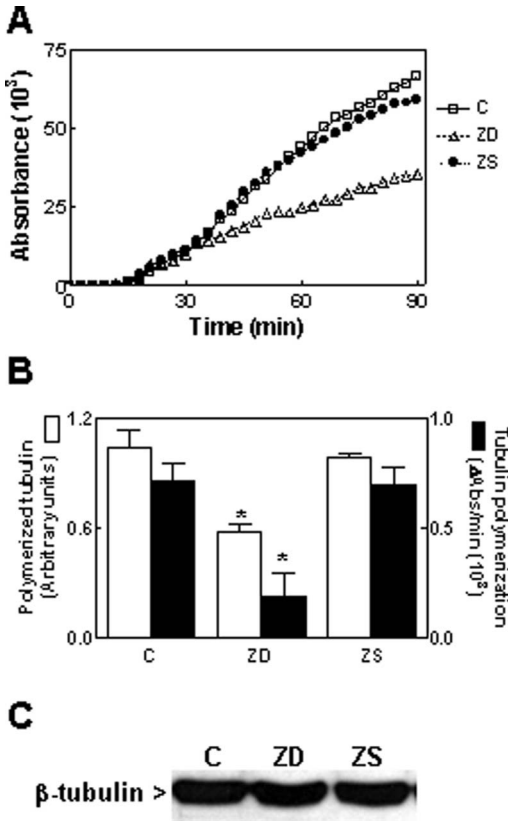


**Figure 3.** Zn deficiency alters tubulin polymerization in human neuroblastoma IMR-32 cells. Cells were incubated for 24 h in control media (C), Zn deficient (ZD) or in Zn supplemented (ZS) media. (A) Typical tubulin polymerization kinetics for C, ZD and ZS cells are shown. (B) The slope in the linear portion of the curves was calculated. The cell content of polymerized tubulin was assessed as previously described in (*19*). After Western blot, results were expressed as the ratio polymerized/total $\beta$-tubulin. Results are shown as means $\pm$ SEM of 4–5 independent experiments. *Significantly lower compared to C and ZS groups ($P < 0.05$, one way ANOVA test). (C) Western blot for $\beta$-tubulin in total fractions. Fig. 3 is adapted from (*19, 20*).

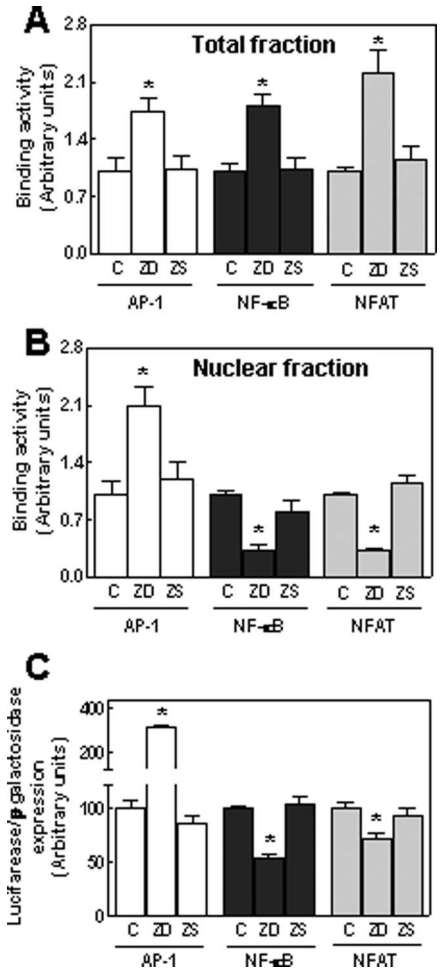


**Figure 4.** Zn deficiency selectively modulates transcription factors AP-1, NF-$\kappa$B and NFAT in human neuroblastoma IMR-32 cells. Total and nuclear fractions were isolated after 24 h of incubating IMR-32 cells in control (C), Zn deficient (ZD) or Zn supplemented (ZS) media. (A) EMSA for AP-1, NF-$\kappa$B and NFAT in total fractions. (B) EMSA for AP-1, NF-$\kappa$B and NFAT in nuclear fractions isolated from cells incubated for 24 h in the corresponding media. After EMSA, the bands of the transcription factor-DNA complexes were quantitated and results are shown as means $\pm$ SEM of 5 independent experiments. *Significantly different compared to C and ZS groups ($P < 0.05$, one way ANOVA test). (C) Cells were co-transfected with a pAP-1-Luc, pNF-$\kappa$B-Luc or p-NFAT-Luc plasmid and a $\beta$-galactosidase plasmid. Twenty four h after the initiation of the transfection, cells were incubated for another 12 h (for AP-1) or 24 h (NF-$\kappa$B and NFAT) in control (C), Zn deficient (ZD) or Zn supplemented (ZS) media. Values are expressed as the ratio luciferase/$\beta$-galactosidase activity. Fig. 4 is adapted from (20–22).

Other family of MAPKs, extracellular signal-regulated kinase 1/2 (ERK1/2), were also studied in Zn deficiency. The MAPKs ERK1/2 are mostly sensitive to mitogenic signals and are involved in processes of cell proliferation through the regulation of the cell cycle. In Zn deficient IMR-32 cells, lower levels of ERK1/2 phosphorylation were observed, in association with a decreased cell proliferation (unpublished results). The incubation of IMR-32 cells with an ERK1/2 inhibitor (PD 98059) causes, within 24 h, a decrease in cell viability, and after 48 h of treatment, apoptosis. These findings indicate the involvement of ERK1/2 in IMR-32 cell proliferation and an association between the inhibition of cell proliferation and the induction of apoptosis in IMR-32 cells. Thus, ERK1/2 inhibition by a decreased Zn availability could be one factor in decreasing neuronal cell proliferation and inducing cell apoptosis.

### *NF-κB*

Transcription factor NF-κB participates in the development of the nervous system through the regulation of neuronal proliferation, differentiation, survival and plasticity (*43*). The activation of NF-κB by different agents and conditions protects neurons from different pro-apoptotic stimuli (reviewed in (*43*)). Accordingly, NF-κB inactivation by proteasome inhibitors triggers apoptosis in different areas of the central nervous system (*44*) and in cultures of IMR-32 cells (*20*). Furthermore, both during development and in the mature brain, the presence of NF-κB in areas of active neurogenesis supports its participation in neuronal proliferation and migration (*45*).

NF-κB modulation is affected by Zn deficiency. NF-κB-DNA binding activity in total cell extracts is higher in Zn deficient IMR-32 cells than in controls (Fig. 4A). The cytosolic events in the NF-κB cascade (IκBα phosphorylation and degradation) are activated in Zn deficient cells (*20*) and this activation is triggered by an increase in cell oxidants (*18*). However, NF-κB-DNA binding activity in nuclear extracts and the NF-κB-dependent gene transactivation are lower in Zn deficient IMR-32 cells compared to control and Zn supplemented cells (Fig. 4B and C) (*20*). Similarly, a low nuclear NF-κB-DNA binding activity has been described for different cell lines including 3T3 fibroblasts (*29*), C6 rat glioma cells (*24*) and a T-lymphoblastoid cell line (HUT-78) (*46*). We have recently described that the alterations in tubulin polymerization occurring secondary to Zn deficiency (*19, 20, 37*) can impair the translocation of the active NF-κB into the nucleus, inhibiting NF-κB-dependent gene expression (*19, 20*). In this regard, we investigated if the stabilization of microtubules with taxol or the stabilization of microfilaments with Jasplakinolide, would prevent Zn deficiency-induced impairment of NF-κB nuclear translocation. While pretreatment with taxol prevented Zn deficiency-induced alterations in NF-κB nuclear transport, microfilament stabilization did not affect NF-κB nuclear transport (*19*). This indicates the requirement of a functional microtubule network for the proper transport and nuclear translocation of NF-κB in neurons. To further investigate this hypothesis we also studied how cytoskeleton disrupting drugs would affect NF-κB nuclear translocation in primary cultures of rat CGN and cortical neurons. Consistent with the results from human neuroblastoma cells, in primary cultures of CGN and cortical neurons, microtubules were necessary for the proper nuclear translocation of NF-κB, since cells treated with either vinblastine or colchicine showed a low nuclear translocation and reduced NF-κB-dependent gene transactivation (*19*).

### *NFAT*

The NFAT signaling cascade can participate in different aspects of neuronal development. NFAT is involved in neuronal plasticity during development and in the mature brain (*47*) and has been shown to protect CGNs from apoptotic death (*48*).

NFAT activation is dependent on calcineurin, a calcium/calmodulin-dependent phosphatase. Calcium levels increased with Zn deficiency (Fig. 1A), and consistently, NFAT activation in total cell extracts, was high in Zn deficient IMR-32 cells (Fig. 4A). The simultaneous incubation of Zn deficient cells with antioxidants, α-lipoic acid and NAC, prevented NFAT activation, suggesting that oxidants are also involved in the initial activation of NFAT in Zn deficiency (*22*). Similarly to NF-κB, NFAT nuclear translocation and dependent gene expression was reduced in Zn deficient IMR-32 cells (Fig. 4B) (*22*). The stabilization of microtubules with taxol reverted Zn deficiency-induced impairment of NFAT nuclear translocation, suggesting a role of microtubules in the nuclear translocation of NFAT (*22*). In support of this mechanism, CGNs need a functional cytoskeleton to transport NFAT into the nucleus, because the treatment of these cells with vinblastine or colchicine inhibited NFAT nuclear transport (*22*).

Thus, the above evidence indicates that a decrease in Zn availability can, through alterations in the microtubule network, impair the nuclear translocation of NF-κB and NFAT in neuronal cells, affecting the expression of genes regulated by these transcription factors.

## ZINC DEFICIENCY AND CELL DEATH BY APOPTOSIS

Zn plays a central role in cell proliferation and death, processes closely interrelated in a balance that will determine the cell's fate. Substantial evidence (*49, 50*), both *in vitro* and *in vivo*, links Zn deficiency to an increased susceptibility of cells to die by apoptosis, affecting critical processes such as embryogenesis (*51*) and immune function (*52*).

In neuronal cells, we found that Zn deficiency induces apoptosis (*23*). Incubation of IMR-32 cells in Zn deficient media leads to a decreased cell viability (Fig. 5A), that is in part due to increased cell death by apoptosis. This is evidenced by caspase-3 activation, an irreversible step in the apoptotic cascade, and increased cytosolic mono and oligonucleosomes, formed secondarily to DNA fragmentation (Fig. 5B). Characterization of cell morphology by the BENA staining (*53*) (Fig. 5C) showed typical apoptotic features, with condensed cytoplasm and nuclei and the presence of apoptotic bodies in Zn deficient cells.

Given the evidence that oxidants and antioxidants can modulate apoptosis, combined with the fact that AP-1 is involved in events ranging from proliferation to apoptotic cell death, we investigated if the $H_2O_2$-driven MAPK and AP-1 activation could be one signal involved in the triggering of neuronal apoptosis in Zn deficiency (*21*). Treatment with catalase prevented Zn deficiency-induced activation of JNK, p38 and AP-1. Caspase-3 activation and DNA fragmentation were elevated in the Zn deficient cells regardless of the absence

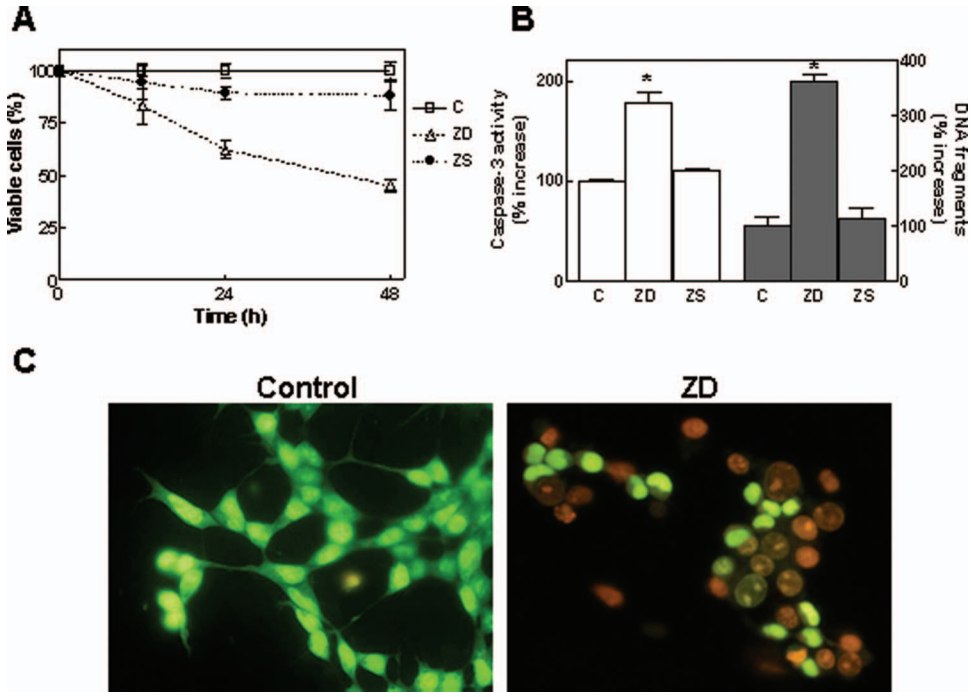


**Figure 5.** Zn deficiency induces cell death by apoptosis in human neuroblastoma IMR-32 cells. (A) Cell viability after 12 h, 24 h or 48 h of incubation in control (C), Zn deficient (ZD) or Zn supplemented (ZS) media. Cell viability was measured by evaluating the exclusion of the Trypan blue dye. (B) Cells were incubated in control (C), Zn deficient (ZD) or Zn supplemented (ZS) media for 24 h, and caspase-3 activity (empty bars) and DNA fragmentation (grey bars) were measured as described in (*21*). Results are shown as means $\pm$ SEM of 4 independent experiments. *Significantly different compared to C and ZS groups ($P < 0.01$, one way ANOVA test). (C) Cells were incubated in control (C) or Zn deficient (ZD) media for 48 h, and apoptosis was assessed by ethidium bromide and acridine orange (BENA) staining (*53*) and evaluated by fluorescent microscopy (magnification $\times$ 40). Fig. 5B is adapted of results from (*21*).

or presence of catalase (*21*). Thus, Zn deficient-induced apoptosis in neuronal cells seems to be independent of $H_2O_2$-mediated activation of AP-1. On the other hand, Zn deficiency inhibited MAPK ERK1/2 phosphorylation which was associated with a decreased cell proliferation (*21*). This is in agreement with recent findings showing that Zn deficiency induces altered receptor tyrosine kinase signaling affecting pro-survival and mitogenic kinase pathways such as RAS $\rightarrow$ ERK and PI3K $\rightarrow$ AKT pathways in 3T3 cells (*49*).

As previously described, Zn deficiency inhibitis NF-$\kappa$B- and NFAT-dependent gene expression (Fig. 4C) (including the anti-apoptotic protein Bcl-2 (*19*)). Both transcription factors are associated with neuronal survival and the inhibition of apoptosis (*43, 48*). In neurons, the pro-survival action of NF-$\kappa$B has been proposed to be mediated through the regulation of NF-$\kappa$B-driven genes encoding anti-apoptotic proteins (Bcl-2, Bcl-xl, IAPs, TRAF1, and TRAF2), antioxidant enzymes, and proteins involved in calcium homeostasis (*43*). NFATc4/NFAT3 protects cerebellum granule cells from apoptotic death (*48*). Accordingly, the inhibition of NF-$\kappa$B and NFAT with lactacystin (*20*) or cyclosporine A (*22*), respectively, induced apoptosis in human IMR-32 cells, suggesting that both cell signals are involved in the protection of IMR-32 cells from apoptosis. Thus, the decreased expression of NF-$\kappa$B- and NFAT-dependent genes could be one of the underlying causes of Zn deficiency-induced neuronal apoptosis.

In summary, and as depicted in Fig. 6, in neuronal cells a decrease in Zn availability is associated with an increase in cellular calcium, a condition of oxidative stress, including elevated levels of $H_2O_2$, and alterations in the cytoskeleton (microtubules and microfilaments) structure and dynamics. Zinc deficiency causes a reduction in cell proliferation and an increased cell death by apoptosis. The elevation of oxidant species leads to the activation of the signaling cascades MAPK/AP1, NF-$\kappa$B and NFAT. However, microtubule disruption impairs NF-$\kappa$B and NFAT nuclear translocation and dependent gene expression. The apoptotic scenario associated with Zn deficiency can not be attributed to the oxidant-induced AP-1 activation. Zn deficiency-induced neuronal apoptotic death can be in part due to alterations in cell proliferation and survival signals, like ERK and AKT (*49*),

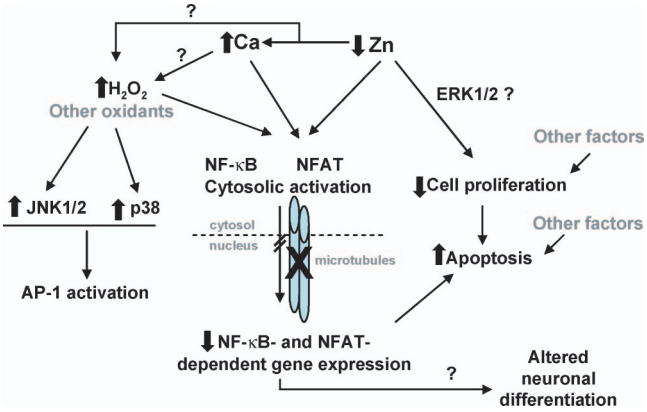


**Figure 6.** Cellular events triggered by a decrease in Zn availability in neuronal cells.

and to the inactivation of the survival pathways NF-κB (*19, 20*) and NFAT (*22*). We hypothesize that, besides the deleterious effects of overt Zn deficiency, subtle variations of extracellular Zn concentrations could constitute a signal involved in the modulation of neuronal development.

## ACKNOWLEDGEMENTS

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