ZINC CROSSTALK WITH SIGNALING PATHWAYS AT A GLANCE

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ABSTRACT

For the first time, zinc (Zn) was identified as a critical micronutrient in 1869 while studying the growth of the fungus *Aspergillus niger*. Subsequently, it was confirmed as such in both the plant and animal kingdoms. Over the years a substantial body of research has demonstrated that the deficiency of zinc in the organism, either due to low dietary intake or impaired absorption in the intestine, leads to abnormal development during embryonic stages and affects the nervous system, hormonal balance, immune system, digestive system, and vascular system in adults. Throughout the lifespan of a living cell, zinc levels fluctuate within a physiological concentration range, playing a crucial role in protein stability and enzyme activity. Extensive work has been conducted in an attempt to elucidate the potential crosstalk between cellular zinc and various signaling pathways. In this review, we provide an overview of the crosstalk between zinc and several signaling pathways, with a particular focus on proliferation and apoptosis.
***Keywords*:** signaling pathways, reactive species, apoptosis, proliferation

Historical perspective on zinc-related research

Vallee and Falchuk (1993) showed that the lack of zinc (Zn) in the organism, either due to low dietary intake or impaired absorption from the intestine, leads to abnormal development during the embryonic stages and affects various systems in adults, including the nervous system, hormonal balance, immunity system, digestive system, and vascular system. These multifaceted interactions of Zn are associated with its presence in nearly every compartment of the cell. While the majority of cellular Zn is distributed throughout the cell, including the nucleus and organelles, only low levels are associated with cell membranes. Studies have shown that most Zn circulates within the cell bound to proteins and anionic complexes (Beyersmann and Haase 2001). Cellular Zn can modulate the activity of phosphodiesterases (PDEs) in a concentration-dependent manner. Specifically, higher concentrations of Zn lead to the activation of the enzyme, while lower concentrations have an inhibitory effect. This phenomenon has been demonstrated through investigations of the binding affinity of cGMP and cAMP to their respective PDEs (Omburo *et al*., 1995; Kovala *et al*., 1997). Conversely, cGMP can inhibit Zn uptake, although the underlying mechanism is not yet fully understood (Haase 2001).

There have been some controversies surrounding the effect of Zn on mitogen-activated protein kinases (MAP kinases). The deficiency of Zn in human and rat cells has been shown to result in a decrease in insulin-like growth factor 1 (IGF-1) and its corresponding gene expression. However, in rat fibroblasts, the phosphorylation levels of IGF-1/2 receptor and its substrates remain unaffected despite alterations in Zn concentration (MacDonald 2000).

Conversely, higher Zn concentrations have been found to stimulate the phosphorylation of protein kinases and the activity of MAP kinases (Hansson 1996; Wu *et al*., 1999). Among the initial substrates of MAP kinases affected by Zn concentration are Jun and ATF-2, mediated through the activation of ERK, JNK, and p38 (Samet *et al*., 1998). Additionally, Zn can stimulate the PI3K (phosphatidylinositol 3-kinase) pathway (Kim *et al*., 2000).

Zinc interference with cell cycle transition

Zinc (Zn) was initially identified as a critical micronutrient in 1869 during the study of the growth of the fungus *Aspergillus niger* and was later confirmed as such in both the plant and animal kingdoms (Prasad 1993). For a considerable time, it was widely accepted that Zn played a pivotal role in cell

proliferation. The absence of Zn interfered with DNA replication by preventing thymidine incorporation, underscoring its importance in the G1/S transition (Chesters *et al*., 1989).

In subsequent years, studies revealed that the absence of Zn could be a limiting factor for transitioning into the S phase until the restriction point in mid-G1. Once the cell overcame this restriction point, it became committed to progressing into the next phase of the cell cycle, regardless of the presence or absence of Zn. In 1999, Chesters and Petrie further elucidated that Zn was required for cells to reenter the G1 phase after completing mitosis (Chesters and Petrie 1999).

The application of modern techniques allowed for the observation of individual cells and their behavior when deprived of only Zn but in the presence of necessary mitogens. Under these specific circumstances, circulating cells faced two possibilities: they could either exit the cell cycle and enter quiescence or stall in the S phase. In cases where the latter occurred, the cell was capable of completing one cycle in the absence of Zn and then reentering the next G1 phase. However, the final S phase was characterized by significantly reduced rates and efficiency, leading to a higher accumulation of DNA damage (Maria *et al*., 2020).

Among the entities susceptible to Zn deprivation were p53 and AP1, two transcription factors crucial for DNA repair mechanisms (Ho and Ames 2002; Yan *et al*., 2008). When cells chose the path of entering quiescence, they exhibited hypoactivity of CDK2, less DNA damage accumulation, and inactivation of p21 (Maria *et al*., 2020). This cell cycle profile contrasted with naturally occurring quiescence, where cells accumulated extensive DNA damage and displayed high p21 activity (Arora *et al*., 2017; Barr *et al*., 2017), confirming that Zn deprivation was the key factor inducing quiescence. The reintroduction of Zn empowered quiescent and S-phase cells to resume their progression through the cell cycle (Maria *et al*., 2020).

Zn – MAPK signaling pathway

Considerable evidence now supports the idea that zinc (Zn) levels within living cells fluctuate within physiological concentrations (Yamasaki *et al*., 2007; Kim *et al*., 2011; Vergnano *et al*., 2014; Que *et al*., 2015; Sanford and Palmer, 2020). Zn primarily serves as a vital biological cofactor, playing a pivotal role in protein stability and enzyme activity (McCall *et al*., 2000).

In the past year, extensive research has been conducted to elucidate the potential crosstalk between cellular zinc and various signaling pathways. One of the initial interactions discovered was with the calcium (Ca) signaling pathway, where higher concentrations of Zn in the cytosol induced a release of

calcium from the endoplasmic reticulum (ER), and vice versa (Qin *et al*., 2011).

Anson *et al*., (2021) demonstrated that, under specific conditions, an increase in Zn concentration within the low micromolar range (40 µM) had no effect on cytosolic Ca levels. During their investigation, they monitored the activation of the mitogen-activated protein kinase (MAPK) pathway and observed increased phosphorylation of extracellular signal-regulated kinase (ERK) in a Zn-specific manner, primarily through the activation of Ras. This activation did not occur by blocking ERK-directed phosphatases, as was previously believed (Ho *et al*., 2008).

Based on their findings, Anson *et al*., (2021) hypothesized that Zn influx might directly activate Ras, leaving its main upstream activator, the epidermal growth factor receptor (EGFR), untouched. This could potentially lead to the phosphorylation of Akt, possibly via phosphoinositide 3-kinase (PI3K). Importantly, this new hypothesis is supported by the fact that Zn administration was conducted under strict conditions involving low concentrations in the range of nanomolar and low micromolar levels, which do not activate stress response signaling pathways, apoptosis pathways, or induce toxicity.

Redox signaling and zinc's role

The definition of redox signaling has undergone multiple revisions by different researchers. However, they all agree on two crucial components: the reactive species involved and the stimulated reactions or pathways. Many well-characterized signaling pathways within cells rely on specific second messengers during their signal transduction. Redox pathways share this characteristic but have a key difference—the secondary messengers in redox signaling can oxidize other molecules not originally part of the redox cascade, sometimes independently of any signaling pathway (Pillay *et al*., 2016).

In most cases, redox reactions induce the oxidation or reduction of cysteine residues. Another notable feature of these reactions is the distance between the reactive species and their target. Peroxides such as H2O2, O-, and OH- are the most important and well-characterized secondary messengers in redox pathways (Forman *et al*., 2014). Studies have demonstrated instances where zinc serves as a secondary messenger and cases where zinc acts as an inhibitor of cysteine oxidation (Maret and Vallee 1998). During the oxidation of target proteins, also known as redox transducers, zinc ions may be released, affecting other proteins involved in various cellular pathways such as apoptosis or proliferation.

One of the transcription factors affected by zinc is Nrf2 (nuclear factor erythroid 2-related factor 2), which, under stress conditions, controls the

transcription of antioxidative compounds within the cell (Itoh *et al*., 1997; Kansanen *et al*., 2013). In the presence of reactive species, zinc ions can alter the molecular structure of Nrf2 repressor Keap1, leading to increased stability of the transcription factor (McMahon *et al*., 2018).

Phosphatases represent another group of proteins that can be targeted for inhibition by zinc. Based on the type of amino acid phosphorylated, phosphatases are divided into three specific groups: 1- PTP (protein tyrosine phosphatase); 2- PSP (protein serine/threonine phosphatase); and 3- DUPS (dual-specificity phosphatase). Among the PTPs inhibited by zinc, even at very low concentrations (nanomolar), is PTEN (phosphatase and tensin homolog), which acts as a tumor suppressor by inhibiting PIP3 (phosphatidylinositol-3,4,5-triphosphate), which, in turn, activates Akt. Consequently, zinc indirectly induces survival mechanisms and proliferation (Yang and Arrizabalaga 2017). An example of zinc regulation in PSPs is the direct binding of zinc with PP2A and lambda serine/threonine PSPs. Both of these enzymes are involved in several signaling pathways that lead to cell proliferation, cell death, and lipid metabolism (Zhuo and Dixon 1997; Seshacharyulu *et al*., 2013).

Regenerate Abnormalities in zinc physiology

Alterations in zinc concentration beyond the physiological range can significantly influence various cellular processes, spanning from proliferation and metabolism to chronic inflammation and the impairment of the immune system, leading to systemic disorders. Non-physiological zinc levels directly disrupt the redox equilibrium, shifting it toward the pro-oxidative side and resulting in an increase in reactive species and total reactive oxygen species (ROS). These elevated ROS levels can interfere with the nuclear translocation of two transcription factors, STAT 1 and 3, thereby inhibiting gene transcription in neurons and causing abnormal brain development (Supasai *et al*., 2017). Oxidative stress induced by zinc deficiency has been observed to decrease cell proliferation by reducing the activity of ERK1/2. Additionally, it has been shown that the activity of JNK, p38, and AP-1 is increased under these conditions (Zago *et al*., 2005).

CONCLUSION

Zinc plays a crucial role in signal transduction and is implicated in pathways that lead to proliferation, cell survival, and apoptosis. Moreover, zinc homeostasis helps maintain the delicate balance between prooxidative and antioxidative molecules, thereby protecting cells from the damaging effects of reactive oxygen species. However, when zinc levels deviate beyond

the physiological range, it has been demonstrated to induce oxidative stress and potentially premature cell death (Huebner and Haase 2021). Despite extensive evidence regarding zinc's role in numerous cellular processes under both normal and abnormal conditions, there is still a need for further research to better elucidate the physiological significance of zinc crosstalk.

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