Zn²⁺ at a cellular crossroads

Xiaomeng Liang, Robert E. Dempski and Shawn C. Burdette*

Zinc is an essential micronutrient for cellular homeostasis. Initially proposed to only contribute to cellular viability through structural roles and non-redox catalysis, advances in quantifying changes in nM and pM quantities of Zn²⁺ have elucidated increasing functions as an important signaling molecule. This includes Zn²⁺-mediated regulation of transcription factors and subsequent protein expression, storage and release of intracellular compartments of zinc quanta into the extracellular space which modulates plasma membrane protein function, as well as intracellular signaling pathways which contribute to the immune response. This review highlights some recent advances in our understanding of zinc signaling.

Address

Worcester Polytechnic Institute, Department of Chemistry and Biochemistry, Worcester, MA 01609-2280, United States

Corresponding author: Burdette, Shawn (scburdette@wpi.edu)

Introduction

Zinc is essential for cellular homeostasis. More specifically, ten percent of all genes code for zinc-binding motifs [1]. Zinc dyshomeostasis can lead to cell death. Once believed to be essential solely for structural and catalytic functions in proteins and enzymes, zinc recently has been recognized as a signaling agent. Specific stimuli are hypothesized to trigger a transient increase in free zinc (Zn²⁺), which subsequently interacts with target molecules in a signal transduction process. In contrast to alkali and alkali earth metal-mediated signaling, the term free zinc does not necessarily denote hydrated metal ions, which adds complexity to these events. Instead Zn²⁺ exists in a weakly bound form that is more accessible than protein-bound zinc [2]. Transient Zn²⁺ increases can be generated by efflux from vesicles (zincosomes) or

changes in cellular redox potential mediated by cytosolic proteins [3]. Metal ion buffering and muffling are the two critical parameters that determine zinc availability and therefore the nature of signaling process [4].

Estimates place the eukaryotic resting free Zn^{2+} concentrations in the picomolar range depending on the cell type [5-7], although quantifying these exceedingly low concentrations remains a significant technical challenge [8,9]. Since the total concentrations of zinc in cells is between 200-300 μM [10], maintaining homeostasis requires complex cellular machinery. This includes transcription factors which are turned on or off by small changes in the cellular concentration of Zn^{2+} as well as proteins that are marked for degradation through the ubiquitination pathway when cellular zinc levels increase for example [11,12]. Both thermodynamic and kinetic models of homeostasis are necessary to understand zinc maintenance. In the thermodynamic model pZn describes the physiological zinc potential [5]. Analogous to pH , the value of pZn is determined by the K_d of the buffering ligand(s) ($\text{pZn} = -\log[\text{Zn}^{2+}]$). Strongly buffered zinc implies that high affinity ligands maintain low free Zn^{2+} concentrations. Under these conditions, a transient increase in Zn^{2+} concentration will return to resting concentrations quickly. When buffering capacity is weak, cells reach a new steady-state Zn^{2+} concentration. To produce lasting Zn^{2+} signals, buffering capacity must be lower since signals would be terminated quickly at high pZn .

Muffling denotes the time-dependent redistribution of internalized Zn^{2+} under non-steady state conditions, and the subsequent return to resting cytosolic concentrations [10,13]. Removing surplus zinc from the cytoplasm by muffling enables cells to increase zinc uptake, despite a lack of buffering capacity. The temporal component of muffling process, as well as the ability for cells to temporarily tolerate non-steady state Zn^{2+} concentrations are important factors in signaling.

Cellular Zinc Entry

Two classes of zinc transporters control the concentration of zinc in the cytosol as well as in intracellular compartments. Zinc Transport (ZnT) proteins function to decrease the cytosolic concentrations, while Zinc- or Iron-regulated transport Proteins (ZIP) increase the cytosolic concentrations [14]. While ZIP transporters are members of the cation diffusion facilitator class of proteins, ZnT proteins are not. Comparison of the crystal structure of YiiP, a bacterial ZnT protein with an *ab initio* model of the human (h) ZIP4 transporter suggests that both protein families have a similar transmembrane metal coordination site [15,16]. Both ZnT and ZIP proteins are expressed on the plasma membrane as well as on the membranes of intracellular organelles. Therefore, both ZnT and ZIP proteins control zinc trafficking between cellular compartments and zinc availability. One of these proposed intracellular compartments, the zinosomes, are loaded with Zn^{2+} , usually by ZnT3. Furthermore, zinosomes may interact with receptors on cell membranes [17,18].

In addition to zinc-specific routes of entry mediated by ZnT and ZIP transport proteins, several ion channels may conduct Zn^{2+} under specific conditions. Under normal resting conditions blood plasma has sub-nM free Zn^{2+} [19]. However, transient increases in extracellular free Zn^{2+} have been observed, most commonly in the synaptic cleft. Under these conditions, some ion channels can use the electrochemical gradient to conduct Zn^{2+} . For example, voltage-gated calcium channels control Ca^{2+} cell entry in response to membrane potential changes [20]. More specifically, the dihydropyridine-sensitive L-type calcium channel (LTCC) contributes to Zn^{2+} import in cortical neurons [21], β -cells in pancreas islet [22], and Zn^{2+} release from the endoplasmic reticulum in mast cells [23]. Ionotropic glutamate receptors (GluRs) are ligand-gated ion channels expressed mainly in the central nervous system (CNS) that mediate most excitatory synaptic transmission in mammalian brains [24]. Various GluR subunits may provide Zn^{2+} entry routes in postsynaptic neurons and astrocytes during neuronal excitation [7,25,26]. In

addition, both acetylcholine receptors (AChR) [27] and transient receptor potential (TRP) channels have been shown to conduct Zn^{2+} [25].

Zinc-Mediated Modulation of Membrane Protein Function

Glutamate-gated NMDA receptors play a crucial role in neuronal communication, synaptic plasticity and memory formation [28,29]. The NMDA receptor is a dimer (GluN1A and GluN2B) where each subunit consists of an amino-terminal domain (ATD), a ligand-binding domain (LBD) and transmembrane domains (TMD) [30]. Upon the requisite stimulus, zinc quanta can be released from zincosomes within presynaptic terminals into the synaptic cleft (Figure 1). The exposed ATD can bind allosterically active ligands including Zn^{2+} found in the extracellular space. The inhibitory role of Zn^{2+} on NMDA receptors was recognized when micromolar Zn^{2+} in cultured hippocampal neurons reduced the amplitude of excitatory postsynaptic potentials by 50% compared to controls [31]. Subsequent analysis revealed that Zn^{2+} inhibits NMDA directly by two different mechanisms. High-affinity Zn^{2+} binding to the ATD of GluN2A subunit stabilized closed-cleft conformations, which reduced channel open probability. Low-affinity binding to the TMD is voltage-dependent and transiently blocks current flow through open channel [32].

The G-protein-coupled zinc sensing receptor (ZnR/GPR39) is expressed in the pancreas, gastrointestinal tract, liver, adipose tissue and the central nervous system. ZnR signaling contributes to cell proliferation, differentiation and/or death. [33-35] Unlike the inhibitory effect on the NMDA receptors upon zinc coordination, ZnR/GPR39 is an activator. Zn^{2+} coordination is mediated through a tridentate Zn^{2+} -binding site on the extracellular domain which is comprised of two histidine and one aspartic acid residue [36]. Zn^{2+} coordination to ZnR triggers Ca^{2+} release via the IP3 signaling pathway [37].

Protein-Mediated Intracellular Zn^{2+} Release

In contrast to extracellular Zn^{2+} signaling which is initiated upon zinc release from an intracellular compartment into the extracellular space, intracellular Zn^{2+} signaling can be

triggered by metal ion release from cytosolic proteins. A transient increase in intracellular Zn^{2+} is dependent upon oxidation of protein ligands since the metal ion is redox-inactive under physiological conditions. Consequently, a redox signal is converted into a Zn^{2+} signal. As an example, metallothioneins (MTs) bind up to seven Zn^{2+} in two thiolate-rich sites. The α -domain (C-terminal), which binds four metal ions, has a higher affinity for Zn^{2+} , while the β -domain (N-terminal) coordinates up to three more labile metal ions [38]. At least four different MTs have been identified including MT-3 that is widely distributed in the CNS [39-41]. Nitric oxide, which has also been identified as an important signaling molecule, can oxidize the cysteine thiolate ligands, releasing a zinc signal [42].

Metal-responsive transcription factor-1 (MTF-1) is a zinc-finger transcription protein which regulates metal-responsive gene expression [43,44]. MTF-1 is the main activator for MT expression as well as select ZnTs [45,46]. MTF-1 contains six Cys_2His_2 zinc fingers (ZFs) which recognize and bind to DNA metal responsive elements (MRE) [47,48]. Zn^{2+} , which can be acquired directly from mobile pools in the cytosol or indirectly by Zn^{2+} release from MTs by either cadmium or oxidative stress, activates MTF-1 upon coordination to ZFs [49,50]. Activated MTF-1 then localizes to the nucleus, binds to the MRE and alters protein expression levels on the transcriptional level [51].

Representative Signaling Events Trigger by Intracellular Zn^{2+} Release

Extracellular stimuli such as hormones, growth factors and cytokines can trigger zinc transients in the cytosol [18]. Transient changes in the intracellular concentration of Zn^{2+} initiate second messenger pathways through early (fast) zinc signaling (EZS) or late zinc signaling (LZS, Figure 2i) [52]. EZS is transcription-independent where the timescale ranges from seconds to minutes. In contrast, LZS requires the transcription of zinc transport proteins with longer lasting effects (hours, Figure 2ii).

Toll-like receptors (TLRs) contribute to the innate immune response by recognizing microbes [53]. TLRs are widely expressed on the surface of immune cells such as dendritic cells and macrophages, as well as on non-immune cells such as fibroblasts and epithelial cells [54-56]. When TLR-4 is activated it oligomerizes and recruits Toll/IL-1 receptor domain-containing

adaptors to initiate signal transduction pathways that culminate in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinases (MAPKs) [57-60]. NF- κ B is a transcription factor which contributes to multiple physiological and pathological conditions, including the apoptosis, carcinogenesis, immune response and inflammation. MAPKs function to phosphorylate serine, threonine and tyrosine. Phosphorylation mediated by MAPKs contributes to initiating the immune response.

Zn²⁺ produces a dual effect on TLR-4 induced cytokine secretion [61]. Stimulation of human leukocytes with lipopolysaccharide (LPS) results in a moderate cytosolic Zn²⁺ increase. Trapping Zn²⁺ with *N,N,N',N'*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) suppresses MAPK stimulation. Zn²⁺-induced MAPK activation occurs within 2 min [62]. LPS can also decrease labile Zn²⁺, and high Zn²⁺ concentrations inhibit TLR-4 signals [63-66]. Zn²⁺ may be involved in TLR-4 signaling, regulating myeloid differentiation primary response gene 88 via EZS, and TIR-domain-containing adapter-inducing interferon- β signaling by modulating basal Zn²⁺ concentrations [67]; however, there is no direct evidence Zn²⁺ activates MAPK. While the kinase might not be the molecular target for Zn²⁺, Zn²⁺ inhibits MAPK phosphatases (MKPs) [68], so the specifics of the putative Zn²⁺ signaling mechanism have yet to be elucidated.

Fc ϵ receptor I (Fc ϵ RI) belongs to the family of multi-chain immune recognition receptors (MIRRs) [69], and is found in mast cells, basophils and eosinophils [70,71]. Upon extracellular stimulus of Fc ϵ RI by immunoglobulin E, a rapid increase in intracellular Zn²⁺, or zinc wave is observed [72]. This zinc wave regulates downstream signaling pathways and enhances the transcription of cytokines and affects several processes, including tyrosine phosphatase activity [18].

Zinc Sparks

Cell cycle arrest at metaphase II (MII) of meiosis is a key step in mammalian oogenesis. Upon fertilization, a series of Ca^{2+} oscillations activate downstream molecular targets [73,74]. The impact of Zn^{2+} on the eukaryotic cell cycle was first recognized three decades ago [75,76], but recently, O'Halloran reported Zn^{2+} uptake by mouse oocytes during maturation [77]. After the onset of Ca^{2+} oscillations, Zn^{2+} -enriched oocytes eject Zn^{2+} into the extracellular milieu in a series of coordinated zinc sparks. The process lasts for hours and facilitates cell cycle resumption. Quantitative maps of the fluxes reveal that zinc sparks originate from vesicles. These vesicles undergo dynamic movement during oocyte maturation and exocytosis upon fertilization [78]. Two zinc transport proteins, ZIP6 and ZIP10, are expressed in the cortex and have been proposed to be responsible for Zn^{2+} accumulation [79]. Cumulus cells also may contribute to the zinc transport network by forming cumulus-oocyte complex [80].

Zinc sparks mediate egg-to embryo transitions [65,77]. Equally, Zn^{2+} may exert a concentration-dependent regulation of meiosis through an interaction with the zinc-binding EM12, a central component of the cytosolic factor (CSF) [81]. EM12 contains a C-terminal zinc-binding region that inhibits the anaphase promoting complex/cyclosome [82-84]. When intracellular Zn^{2+} reaches a threshold concentration, coordination to the zinc binding region activates APC/C inhibitor EM12, which leads to CSF-mediated MII arrest. After activation, zinc sparks reduce both cellular zinc and EM12 activity, which drives egg activation [81].

Conclusions

Zn^{2+} signaling is mediated by transient changes in free zinc. Extracellular Zn^{2+} signaling can take place in extracellular environment such as the synaptic cleft where Zn^{2+} released from the presynaptic cleft modulates protein function on postsynaptic neurons. Intracellular Zn^{2+} signaling

has been observed in many cell types and is generated in the cytosol from the extracellular space or from intracellular Zn^{2+} stores. Cytosolic proteins such as metallothioneins may also contribute to the Zn^{2+} signals through redox chemistry. The import, export and trafficking of Zn^{2+} by proteins all contribute to a cells capacity to generated, utilize and terminate signals. The ongoing efforts to study these various processes are vital to mapping signal transduction pathways mediated by Zn^{2+} .

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References and recommended reading

- of special interest

- of outstanding interest

1. Andreini C, Banci L, Bertini I, Rosato A: **Counting the zinc-proteins encoded in the human genome.** *J Proteome Res* 2006, **5**:196-201.

- 2. Wellenreuther G, Cianci M, Tucoulou R, Meyer-Klaucke W, Haase H: **The ligand environment of zinc stored in vesicles.** *Biochem Biophys Res Commun* 2009, **380**:198-203.

- X-ray absorption techniques are utilized to characterize the nature of vesicular (often called free) Zn^{2+} . Measurements indicate a coordination environment including nitrogen, oxygen and sulfur donors.

3. Fukada T, Kambe T: *Zinc Signals in Cellular Functions and Disorders*. Springer, (2014).

4. Wang Z, Tymianski M, Jones OT, Nedergaard M: **Impact of cytoplasmic calcium buffering on the spatial and temporal characteristics of intercellular calcium signals in astrocytes.** *J Neurosci* 1997, **17**:7359-7371.

- 5. Krężel A, Maret W: **Zinc-buffering capacity of a eukaryotic cell at physiological pZn.** *J Biol Inorg Chem* 2006, **11**:1049-1062.

- A detailed picture of Zn^{2+} concentrations and binding sites in human colon cancer cells is provided. The findings highlight the importance of metallothionein as a buffer for intracellular Zn^{2+} .

6. Maret W: **Metals on the move: zinc ions in cellular regulation and in the coordination dynamics of zinc proteins.** *Biomaterials* 2011, **24**:411-418.

7. Sensi SL, Canzoniero LM, Yu SP, Ying HS, Koh J-Y, Kerchner GA, Choi DW: **Measurement of intracellular free zinc in living cortical neurons: routes of entry.** *J Neurosci* 1997, **17**:9554-9564.

8. Park JG, Qin Y, Galati DF, Palmer AE: **New sensors for quantitative measurement of mitochondrial Zn^{2+} .** *ACS Chem Biol* 2012, **7**:1636-1640.

- 9. Qin Y, Miranda JG, Stoddard CI, Dean KM, Galati DF, Palmer AE: **Direct comparison of a genetically encoded sensor and small molecule indicator: implications for quantification of cytosolic Zn^{2+} .** *ACS Chem Biol* 2013, **8**:2366-2371.

•• Small molecule fluorescent probes are popular for measuring intracellular metal ion concentrations. Comparison with protein-based sensors highlights some of the challenges of using small molecule probes that can perturb actual behavior.

10. Maret W: **Analyzing free zinc(II) ion concentrations in cell biology with fluorescent chelating molecules.** *Metallomics* 2015, **7**:202-211.

11. Mao X, Kim B-E, Wang F, Eide DJ, Petris MJ: **A histidine-rich cluster mediates the ubiquitination and degradation of the human zinc transporter, hZIP4, and protects against zinc cytotoxicity.** *J Biol Chem* 2007, **282**:6992-7000.

•• 12. Outten CE, O'Halloran TV: **Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis.** *Science* 2001, **292**:2488-2492.

•• Seminal study demonstrating the extremely tight regulation of intracellular Zn²⁺ in E. coli. The investigation contradicts the previous metalloregulatory dogma that proteins acquire Zn²⁺ from a pool of free metal ions.

• 13. Colvin RA, Holmes WR, Fontaine CP, Maret W: **Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis.** *Metallomics* 2010, **2**:306-317.

• An important primer on the thermodynamic and kinetic principles governing Zn²⁺ homeostasis and signaling.

14. Dempski RE: **The cation selectivity of the ZIP transporters.** *Curr Top Membr* 2012, **69**:221-245.

15. Antala S, Ovchinnikov S, Kamisetty H, Baker D, Dempski RE: **Computational modeling and functional studies provide a structural scaffold for the zinc transporter hZIP4.** *J Biol Chem* 2015, jbc. M114. 617613.

16. Lu M, Fu D: **Structure of the zinc transporter YiiP.** *Science* 2007, **317**:1746-1748.

17. Maret W: **Zinc biochemistry: from a single zinc enzyme to a key element of life.** *Adv Nutr* 2013, **4**:82-91.

•• 18. Yamasaki S, Sakata-Sogawa K, Hasegawa A, Suzuki T, Kabu K, Sato E, Kurosaki T, Yamashita S, Tokunaga M, Nishida K: **Zinc is a novel intracellular second messenger.** *J Cell Biol* 2007, **177**:637-645.

•• An extracellular stimulus leads to an increase in intracellular Zn²⁺. The generation of this "zinc wave" has the hallmarks of second messengers, and establishes Zn²⁺ as an important signaling agent.

19. Magneson G, Puvathingal J, Ray W: **The concentrations of free Mg²⁺ and free Zn²⁺ in equine blood plasma.** *J Biol Chem* 1987, **262**:11140-11148.
20. Van Petegem F, Minor Jr D: **The structural biology of voltage-gated calcium channel function and regulation.** *Biochem Soc Trans* 2006, **34**:887.
21. Kerchner GA, Canzoniero LM, Yu SP, Ling C, Choi DW: **Zn²⁺ current is mediated by voltage-gated Ca²⁺ channels and enhanced by extracellular acidity in mouse cortical neurones.** *J Physiol* 2000, **528**:39-52.
22. Priel T, Aricha-Tamir B, Sekler I: **Clioquinol attenuates zinc-dependent β -cell death and the onset of insulinitis and hyperglycemia associated with experimental type I diabetes in mice.** *Eur J Pharmacol* 2007, **565**:232-239.
23. Yamasaki S, Hasegawa A, Hojyo S, Ohashi W, Fukada T, Nishida K, Hirano T: **A novel role of the L-type calcium channel α 1D subunit as a gatekeeper for intracellular zinc signaling: zinc wave.** *PLoS One* 2012, **7**:e39654.
24. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R: **Glutamate receptor ion channels: structure, regulation, and function.** *Pharmacological reviews* 2010, **62**:405-496.
25. Bouron A, Oberwinkler J: **Contribution of calcium-conducting channels to the transport of zinc ions.** *Pflügers Arch EJP* 2014, **466**:381-387.
26. Weiss JH, Sensi SL: **Ca²⁺-Zn²⁺ permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration.** *Trends Neurosci* 2000, **23**:365-371.
27. Ragozzino D, Giovannelli A, Degasperis V, Eusebi F, Grassi F: **Zinc permeates mouse muscle ACh receptor channels expressed in BOSC 23 cells and affects channel function.** *J Physiol* 2000, **529**:83-91.
28. Danysz W, Parsons CG: **Alzheimer's disease, β -amyloid, glutamate, NMDA receptors and memantine—searching for the connections.** *British J Pharmacol* 2012, **167**:324-352.
29. Paoletti P, Bellone C, Zhou Q: **NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease.** *Nat Rev Neurosci* 2013, **14**:383-400.
30. Karakas E, Furukawa H: **Crystal structure of a heterotetrameric NMDA receptor ion channel.** *Science* 2014, **344**:992-997.
31. Mayer M, Vyklicky L: **The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus.** *J Physiol* 1989, **415**:351-365.

32. Amico-Ruvio SA, Murthy SE, Smith TP, Popescu GK: **Zinc effects on NMDA receptor gating kinetics.** *Biophys J* 2011, **100**:1910-1918.

33. Cohen L, Azriel-Tamir H, Arotsker N, Sekler I, Hershfinkel M: **Zinc sensing receptor signaling, mediated by GPR39, reduces butyrate-induced cell death in HT29 colonocytes via upregulation of clusterin.** *PLoS One* 2012, e35482.

34. Cohen L, Sekler I, Hershfinkel M: **The zinc sensing receptor, ZnR/GPR39, controls proliferation and differentiation of colonocytes and thereby tight junction formation in the colon.** *Cell Death Dis* 2014, **5**:e1307.

35. Popovics P, Stewart AJ: **GPR39: a Zn^{2+} -activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions.** *Cell Mol Life Sci* 2011, **68**:85-95.

36. Storjohann L, Holst B, Schwartz TW: **Molecular mechanism of Zn^{2+} agonism in the extracellular domain of GPR39.** *FEBS Lett* 2008, **582**:2583-2588.

37. Asraf H, Salomon S, Nevo A, Sekler I, Mayer D, Hershfinkel M: **The ZnR/GPR39 interacts with the CaSR to enhance signaling in prostate and salivary epithelia.** *J Cell Physiol* 2014, **229**:868-877.

• 38. Babu CS, Lee Y-M, Dudev T, Lim C: **Modeling Zn^{2+} Release From Metallothionein.** *J Phys Chem A* 2014, **118**:9244-9252.

• Modeling techniques are introduced as a powerful methodology to understand how Zn^{2+} can be liberated from differential binding sites in metallothionein. These processes are important to maintaining homeostasis and possibly to generating Zn^{2+} signals.

39. Garrett SH, Sens MA, Todd JH, Somji S, Sens DA: **Expression of MT-3 protein in the human kidney.** *Toxicol Lett* 1999, **105**:207-214.

40. Quaife CJ, Findley SD, Erickson JC, Froelick GJ, Kelly EJ, Zambrowicz BP, Palmiter RD: **Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia.** *Biochemistry* 1994, **33**:7250-7259.

41. Sallinen P, Saarela S, Ilves M, Vakkuri O, Leppäluoto J: **The expression of MT 1 and MT 2 melatonin receptor mRNA in several rat tissues.** *Life Sci* 2005, **76**:1123-1134.

42. Thambiayya K, Wasserloos KJ, Pitt BR: **Central role for metallothionein (MT) in Zn dependent nitric oxide (NO) mediated resistance to LPS-induced apoptosis in sheep pulmonary artery endothelial cells (SPAEC).** *FASEB J* 2011, **25**:1100.1102.

43. Giedroc DP, Chen X, Apuy JL: **Metal response element (MRE)-binding transcription factor-1 (MTF-1): structure, function, and regulation.** *Antioxid Redox Signal* 2001, **3**:577-596.

44. Saydam N, Georgiev O, Nakano MY, Greber UF, Schaffner W: **Nucleo-cytoplasmic trafficking of metal-regulatory transcription factor 1 is regulated by diverse stress signals.** *J Biol Chem* 2001, **276**:25487-25495.
45. Langmade SJ, Ravindra R, Daniels PJ, Andrews GK: **The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene.** *J Biol Chem* 2000, **275**:34803-34809.
46. Lu Y-J, Liu Y-C, Lin M-C, Chen Y-T, Lin L-Y: **Coordinative modulation of human zinc transporter 2 gene expression through active and suppressive regulators.** *J Nutr Biochem* 2015, **26**:351-359.
47. Dalton TP, Bittel D, Andrews GK: **Reversible activation of mouse metal response element-binding transcription factor 1 DNA binding involves zinc interaction with the zinc finger domain.** *Mol Cell Biol* 1997, **17**:2781-2789.
48. Koizumi S, Suzuki K, Ogra Y, Gong P, Otuska F: **Roles of zinc fingers and other regions of the transcription factor human MTF-1 in zinc-regulated DNA binding.** *J Cell Physiol* 2000, **185**:464-472.
49. Dalton TP, Li Q, Bittel D, Liang L, Andrews GK: **Oxidative Stress Activates Metal-responsive Transcription Factor-1 Binding Activity** *J Biol Chem* 1996, **271**:26233-26241.
50. Zhang B, Georgiev O, Hagmann M, Günes Ç, Cramer M, Faller P, Vasák M, Schaffner W: **Activity of metal-responsive transcription factor 1 by toxic heavy metals and H₂O₂ in vitro is modulated by metallothionein.** *Mol Cell Biol* 2003, **23**:8471-8485.
51. Günther V, Lindert U, Schaffner W: **The taste of heavy metals: gene regulation by MTF-1.** *Biochim Biophys Acta* 2012, **1823**:1416-1425.
52. Hirano T, Murakami M, Fukada T, Nishida K, Yamasaki S, Suzuki T: **Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule.** *Adv Immunol* 2008, **97**:149-176.
53. Kawai T, Akira S: **TLR signaling.** *Cell Death Differ* 2006, **13**:816-825.
54. Kim K-W, Cho M-L, Lee S-H, Oh H-J, Kang C-M, Ju JH, Min S-Y, Cho Y-G, Park S-H, Kim H-Y: **Human rheumatoid synovial fibroblasts promote osteoclastogenic activity by activating RANKL via TLR-2 and TLR-4 activation.** *Immunol Lett* 2007, **110**:54-64.
55. Krutzik SR, Tan B, Li H, Ochoa MT, Liu PT, Sharfstein SE, Graeber TG, Sieling PA, Liu Y-J, Rea TH: **TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells.** *Nat Med* 2005, **11**:653-660.
56. Vora P, Youdim A, Thomas LS, Fukata M, Tesfay SY, Lukasek K, Michelsen KS, Wada A, Hirayama T, Arditi M: **β-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells.** *J Immunol* 2004, **173**:5398-5405.

57. Huang H, Zhang Z, Cao C, Wang N, Liu F, Peng J, Ren X, Qian J: **The TLR4/NF- κ B signaling pathway mediates the growth of colon cancer.** *Eur Rev Med Pharmacol Sci* 2014, **18**:3834-3843.
58. Lu Y-C, Yeh W-C, Ohashi PS: **LPS/TLR4 signal transduction pathway.** *Cytokine* 2008, **42**:145-151.
59. Saitoh Si, Akashi S, Yamada T, Tanimura N, Kobayashi M, Konno K, Matsumoto F, Fukase K, Kusumoto S, Nagai Y: **Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization.** *Int Immunol* 2004, **16**:961-969.
60. Xu X, Yin P, Wan C, Chong X, Liu M, Cheng P, Chen J, Liu F, Xu J: **Punicalagin inhibits inflammation in LPS-induced RAW264. 7 macrophages via the suppression of TLR4-mediated MAPKs and NF- κ B activation.** *Inflammation* 2014, **37**:956-965.
61. Haase H, Rink L: **Functional significance of zinc-related signaling pathways in immune cells.** *Ann Rev Nutr* 2009, **29**:133-152.
62. Haase H, Ober-Blöbaum JL, Engelhardt G, Hebel S, Heit A, Heine H, Rink L: **Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes.** *J Immunol* 2008, **181**:6491-6502.
63. Heyninck K, Beyaert R: **The cytokine-inducible zinc finger protein A20 inhibits IL-1-induced NF- κ B activation at the level of TRAF6.** *FEBS Lett* 1999, **442**:147-150.
64. Jin J, Zeng H, Schmid KW, Toetsch M, Uhlig S, Mörröy T: **The zinc finger protein Gfi1 acts upstream of TNF to attenuate endotoxin-mediated inflammatory responses in the lung.** *Eur J Immunol* 2006, **36**:421-430.
65. Rink L, Haase H: **Zinc homeostasis and immunity.** *Trends Immunol* 2007, **28**:1-4.
66. Thambiayya K, Wasserloos KJ, Huang Z, Kagan VE, Croix CMS, Pitt BR: **LPS-induced decrease in intracellular labile zinc, [Zn]_i, contributes to apoptosis in cultured sheep pulmonary artery endothelial cells.** *Am J Physiol Lung Cell Mol Physiol* 2011, **300**:L624-L632.
67. Brieger A, Rink L, Haase H: **Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions.** *J Immunol* 2013, **191**:1808-1817.
68. Ho Y, Samarasinghe R, Knoch ME, Lewis M, Aizenman E, DeFranco DB: **Selective inhibition of MAPK phosphatases by zinc accounts for ERK1/2-dependent oxidative neuronal cell death.** *Mol Pharmacol* 2008, **74**:1141.
69. Wilson BS, Pfeiffer JR, Oliver JM: **Observing Fc ϵ R1 signaling from the inside of the mast cell membrane.** *J Cell Biol* 2000, **149**:1131-1142.

70. Eiseman JBB: **Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases.** *Nature* 1992, **355**:78-80.
71. Sihra BS, Kon OM, Grant JA, Kay AB: **Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: Relationship to total serum IgE concentrations.** *J Allergy Clin Immunol* 1997, **99**:699-706.
72. Turner H, Kinet J-P: **Signalling through the high-affinity IgE receptor FcεRI.** *Nature* 1999, **402**:24-30.
73. Kinsey WH: **Intersecting roles of protein tyrosine kinase and calcium signaling during fertilization.** *Cell Calcium* 2013, **53**:32-40.
74. Miao Y-L, Stein P, Jefferson WN, Padilla-Banks E, Williams CJ: **Calcium influx-mediated signaling is required for complete mouse egg activation.** *Proc Natl Acad Sci USA* 2012, **109**:4169-4174.
75. Powell M, Davies M, Francis D: **The Influence of Zinc on the Cell Cycle in the Root Meristem of a Zinc-Tolerant and a Non-Tolerant Cultivar of Festuca Rubra L** *New Phytol* 1986, **102**:419-428.
76. Powell M, Davies M, Francis D: **Effects of Zinc on Cell, Nuclear and Nucleolar Size, and on RNA and Protein Content in the Root Meristem of a Zinc Tolerant and a Non-Tolerant Cultivar of Festuca Rubra L** *New Phytol* 1986, **104**:671-679.
77. Kim AM, Bernhardt ML, Kong BY, Ahn RW, Vogt S, Woodruff TK, O'Halloran TV: **Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs.** *ACS Chem Biol* 2011, **6**:716-723.
- 78. Que EL, Bleher R, Duncan FE, Kong BY, Gleber SC, Vogt S, Chen S, Garwin SA, Bayer AR, Dravid VP: **Quantitative mapping of zinc fluxes in the mammalian egg reveals the origin of fertilization-induced zinc sparks.** *Nat Chem* 2015, **7**:130-139.
- A combination of techniques are utilized to elucidate the origins of Zn²⁺ sparks, which are essential to the conversion of a fertilized egg into an embryo. Zn²⁺ was found to be compartmentalized in vesicles that undergo exocytosis responsible for the observed sparks.
79. Kong B, Duncan F, Que E, Kim A, O'Halloran T, Woodruff T: **Maternally-derived zinc transporters ZIP6 and ZIP10 drive the mammalian oocyte-to-egg transition.** *Mol Hum Reprod* 2014, gau066.
80. Lisle RS, Anthony K, Randall M, Diaz F: **Oocyte-cumulus cell interactions regulate free intracellular zinc in mouse oocytes.** *Reproduction* 2013, **145**:381-390.

81. Bernhardt ML, Kong BY, Kim AM, O'Halloran TV, Woodruff TK: **A zinc-dependent mechanism regulates meiotic progression in mammalian oocytes.** *Biol Reprod* 2012, **86**:114.
82. Kim AM, Vogt S, O'Halloran TV, Woodruff TK: **Zinc availability regulates exit from meiosis in maturing mammalian oocytes.** *Nat Chem Biol* 2010, **6**:674-681.
83. Li M, Zhang P: **The function of APC/CCdh1 in cell cycle and beyond.** *Cell Div* 2009, **4**:2.
84. Shoji S, Muto Y, Ikeda M, He F, Tsuda K, Ohsawa N, Akasaka R, Terada T, Wakiyama M, Shirouzu M: **The zinc-binding region (ZBR) fragment of Emi2 can inhibit APC/C by targeting its association with the coactivator Cdc20 and UBE2C-mediated ubiquitylation.** *FEBS Open Bio* 2014, **4**:689-703.