HHS Public Access

Author manuscript

Curr Med Chem. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Curr Med Chem. 2015 ; 22(10): 1248–1257.

Zinc-Permeable Ion Channels: Effects on Intracellular Zinc Dynamics and Potential Physiological/Pathophysiological Significance

Koichi Inoue, **Zaven O'Bryant**, and **Zhi-Gang Xiong**

Neuroscience Institute, Morehouse School of Medicine, Atlanta, USA

Abstract

Zinc (Zn^{2+}) is one of the most important trace metals in the body. It is necessary for the normal function of a large number of proteins including enzymes and transcription factors. While extracellular fluid may contain up to micromolar Zn^{2+}, intracellular Zn^{2+} concentration is generally maintained at a subnanomolar level; this steep gradient across the cell membrane is primarily attributable to Zn^{2+} extrusion by Zn^{2+} transporting systems. Interestingly, systematic investigation has revealed that activities, previously believed to be dependent on calcium (Ca^{2+}), may be partially mediated by Zn^{2+}. This is also supported by new findings that some Ca^{2+}-permeable channels such as voltage-dependent calcium channels (VDCCs), N-methyl-D-aspartate receptors (NMDA), and amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPA-Rs) are also permeable to Zn^{2+}. Thus, the importance of Zn^{2+} in physiological and pathophysiological processes is now more widely appreciated. In this review, we describe Zn^{2+}-permeable membrane molecules, especially Zn^{2+}-permeable ion channels, in intracellular Zn^{2+}dynamics and Zn^{2+} mediated physiology/pathophysiology.

Keywords

calcium; fluorescence imaging; ion channel; zinc

Introduction

Following iron (Fe^{2+}), Zn^{2+} is the second most abundant trace metal in human and animals. Like other metals, Zn^{2+} is involved in various cellular processes contributing to normal physiology. Enzymes that mediate important cellular processes, for which Zn^{2+} is required, number more than 200 [1-4]. It is speculated that approximately 10% of proteins utilize Zn^{2+} to maintain their structure and/or function [5, 6]. Therefore, either a deficiency or excess of Zn^{2+} is expected to be detrimental. In fact, 23% of the world's population has been estimated to be Zn^{2+} deficient; this deficiency may cause illness such as pneumonia and diarrhea [7, 8]. Indispensable in maintaining membrane potential, especially in excitable cells, energy-dependent Na^+/K^+-pumps generate a 10 to 30-fold gradient for Na^+ and K^+

Send correspondence to: Koichi Inoue (kinoue@msm.edu) or Zhi-Gang Xiong (zxiong@msm.edu).

Conflict of interest

The authors confirm that there is no conflict of interest.

ions [9, 10]. In comparison, the intracellular concentration of Zn^{2+} is only about a thousandth of the extracellular concentration [11-13]. Therefore, effective homeostatic mechanisms must exist for zinc dynamics. Though unique among other ions Zn^{2+} is similar to Ca^{2+}. Ca^{2+} is one of the most investigated metal ions because of its diverse functions in physiological/pathophysiological processes, its widespread abundance, and its high concentration gradient across cellular membranes [14-16]. While intracellular Ca^{2+} dynamics are well established, it is less so for Zn^{2+}. Recent advances in the studies of Zn^{2+} permeable channels and Zn^{2+} transporters have increased our understanding of cellular mechanism in regulating intracellular zinc homeostasis. This review will focus on the role of Zn^{2+}-permeable ion channels in intracellular Zn^{2+} dynamics and cellular physiology/ pathophysiology.

Physical and chemical properties of Zn^{2+}

Zn^{2+} is a very common element distributed in Earth's outer crust and is handily smelted and manufactured due to its low melting point [17, 18]. The International Union of Pure and Applied Chemistry definition states that Zn^{2+}, like cadmium, is regarded as a typical element in which the d-block of electron shell is filled with electrons [19]. Therefore, like cadmium, there is no unpaired electron in the reactive d-block, resulting in less reactivity to redox reaction compared to other divalent metal ions such as iron and cobalt, which are listed in the same period of the periodic table [20]. Considering that the redox reaction is constantly occurring in cells, less reactivity is beneficial so that molecules, such as enzymes, can perform their functions more efficiently. Zn^{2+} is also utilized for acid-base reactions and as a transition metal it is classified as “borderline” for its polarity [21]. Its polarity limits coordination with nitrogen, oxygen and sulfur, which are regarded as soft bases.

Zn^{2+} has been known to be involved in a large number of cellular processes as described below and most commonly it plays a role through its interaction with proteins. Among amino acids, the imidazole functional group of histidine, the carbonyl oxygen of aspartate and glutamate, and the thiol functional group of cysteine are prevalent molecules facilitating Zn^{2+} coordination [5, 22]. With such large variations of coordination, it is of no surprise that Zn^{2+} has a broad spectrum of functions. The functional role of Zn^{2+} in proteins can be classified into two main categories: structural and catalytic. Structurally, Zn^{2+} is normally surrounded by four amino acids preventing solvent interaction. Motifs, such as Zn^{2+} fingers, facilitate not only the stabilization of peptides, but also structural maintenance of the whole protein by mutual interaction with multiple local contextures [1, 4, 5]. In catalytic sites, Zn^{2+} is positioned in active center of the catalytic reaction. Solvents such as water occupy one ligation site of the catalytic Zn^{2+} [5]. The Zn^{2+} metalloenzymes include the family of nucleases, superoxide dismutases, and carbonic anhydrase II (see below) [4].

Physiologic and pathophysiolologic functions of Zn^{2+}

Zn^{2+} is an essential factor for growth of higher organisms [23]. Its importance for growth, first established in plants in 1869 [24], has been revealed across many species [23, 25]. In humans, gonadal dysfunction and growth retardation, seen in the Middle East during the 1960's, was found to be mediated by Zn^{2+}deficiency [26]. Meanwhile, various enzymes

were found to act in a Zn^{2+}-dependent manner and the pathophysiological symptoms related to the dysfunction of those enzymes have been described [13, 27-30]. A well recognizable pathophysiology associated with Zn^{2+} dependent enzymes, acrodermatitis enteropathica, is especially egregious [31]. It is characterized by initial skin lesions (e.g. eruption, erosion), subsequent intestinal disorders (e.g. diarrhea) among weaning infants, and growth and mental retardation. Recent reports suggest that high doses of Zn^{2+} can improve clinical outcomes in both inherited and acquired cases of acrodermatitis enteropathica, as some cases implicated ZIP4, one of the Zn^{2+} transporters, as an aberrant gene [32, 33]. Abnormal zinc homeostasis has also been shown to be involved in other pathophysiological conditions. Insults that result from traumatic brain injury, brain ischemia and epilepsy, for example, contribute to excessive Zn^{2+} levels [34-36]. Dysfunctional Zn^{2+} regulation in the mitochondria, leads to a disruption of cell metabolism [37-39]. The energy-generating mitochondrial oxidation-reduction reaction, on the other hand, requires the presence of intracellular Zn^{2+}; mitochondrial complexes are not functional when Zn^{2+} is chelated, essentially abating ATP generation [40].

Zn^{2+} is relatively less toxic compared to other metals and Zn^{2+} poisoning rarely occur through normal dietary intake. However, absorption of Zn^{2+} through fumes, in gaseous form, and contamination from Zn^{2+}-coated containers may lead to abnormally high concentrations of Zn^{2+} within different organ systems, which may result in disorders of nervous system, intestinal and renal systems [41]. Presently, over-the-counter oral supplementation of vitamins and minerals are common. However, excessive supplementation or iatrogenic errors may cause Zn^{2+} poisoning [42, 43].

Intense research of Zn^{2+} has been conducted in the nervous system although Zn^{2+} is important in other organ systems as well. One reason for systematic study of the nervous system is that neurologic symptoms are often produced by Zn^{2+}-related disorders [26, 41, 44]. Different from other organ systems, the nervous system is surrounded by cerebrospinal fluid in which the Zn^{2+} concentration is much lower than the surrounding interstitial fluid [45-47]. Interestingly, neurons can release Zn^{2+} from their synaptic terminals [48, 49]. Low physiologic concentrations and steep gradients of Zn^{2+} surrounding cells may indicate a tight control mechanism of which Zn^{2+} plays important regulatory role in neuronal functions.

Intracellular Zn^{2+} balance and biochemistry

Over 90% of Zn^{2+} is thought to be bound tightly to intracellular molecules; the remaining intracellular Zn^{2+} is labile [1]. Homeostasis of intracellular Zn^{2+} dynamics generally include the following processes: (1) Zn^{2+} incorporation into intracellular organelles and molecules such as mitochondria and metallothioneins (MTs) [50]; (2) Zn^{2+} extrusion into extracellular space or secretion by vesicles [2, 36]; and (3) Zn^{2+} is transported by Zn^{2+} transporters and Zn^{2+} -permeable ion channels (Figure 1). The existence of Na^{+}/Zn^{2+} and Ca^{2+}/Zn^{2+} exchangers are suggested, but have not been identified at the molecular level [51, 52]. Although recent works have uncovered the importance of extracellular Zn^{2+}, for example, in regulating Zn^{2+} -potentiated ectoenzymes [53-55], in Zn^{2+} -sensing receptors, and in Zn^{2+} -

activated channels [56-58], intracellular Zn^{2+} is still considered to play a leading part in cellular functions.

MTs are small molecules, which have about 70 amino acids; many of them contain about twenty cysteine residues that can trap up to seven Zn^{2+} ions through metal-thiolate bonds [59, 60]. The binding or release of Zn^{2+} ions is regulated by redox status and intracellular pH. Besides MTs, Zn^{2+} is sequestered within the cytoplasm by enzymes and transcription factors. Another main system to maintain intracellular Zn^{2+} levels is the elimination of cytoplasmic Zn^{2+} constituents. In this regard, a number of molecules for Zn^{2+} elimination have been recently cloned [61-63]. Zrt- and Irt- like proteins (ZIPs), which mainly transport Zn^{2+} into cells, and the family of Zn^{2+} transporters (ZnTs), which transport Zn^{2+} out of cytoplasm, seem to work independently within different tissues, and have varied intracellular distribution and transport activity. Some reports have shown that these molecules are involved in human diseases. Although important in Zn^{2+} homeostasis, these transporters are beyond the scope of this review. Readers may obtain valuable information on the subject from other reviews [63-66].

Zn^{2+} is essential for many enzymes which are fundamental to normal cell function. One noteworthy enzyme, carbonic anhydrase II, which catalyzes the reversible hydration of carbonic dioxide, was first to be found as dependent on Zn^{2+} for its activity [4, 67, 68]. These reports were the first to demonstrate important physiological functions of Zn^{2+}. Interestingly, one newly uncovered pathophysiological aspect of Zn^{2+} is that it may act on amyloid precursor protein (APP) and play a role in Alzheimer's disease [69]. APP excretes irons from cytoplasm as iron transporter and also works as ferroxidase, which oxidizes Fe^{2+} to Fe^{3+}. It interacts with ferroportin, which binds to oxidized iron, to facilitate iron transport. Extracellular Zn^{2+}, which is involved in aggregated β-amiloid, is suggested to inhibit the ferroxidase activity, resulting in intracellular iron retention [69].

Zn^{2+} -permeable channels

Zn^{2+} influx across the cellular membrane, through ion channels, is a key factor for the increase of intracellular Zn^{2+} concentration and activation of zinc signaling pathways. The activities of various channels are, in turn, modified by Zn^{2+}. For example, NMDA and γ-aminobutric acid – type A ($GABA_A$) receptor-gated channels are sensitive to Zn^{2+} inhibition, which results in reduced current flux [70-72]. While the activities of many ion channels are modified by Zn^{2+}, some cation-permeable channels are reported to be Zn^{2+} permeable. For example, recent studies have demonstrated that voltage-gated Ca^{2+} channels and Ca^{2+}-permeable ionotropic glutamate receptors conduct Zn^{2+} (see section Voltage-dependent Ca^{2+} channels). It has also been established that Zn^{2+} flux plays an important role in physiological and pathophysiological processes such as long term potentiation and ischemic stress [62, 73, 74]. Here, we provide an overview of Zn^{2+} -conducting ion channels and their potential functions in physiological/pathophysiological processes. The Zn^{2+} -permeable channels are summarized in Table 1.

Voltage-dependent Ca^{2+} channels (VDCCs)

VDCCs are expressed mainly in excitable cells such as muscle and neuronal cells and contribute to cellular Ca^{2+} homeostasis [75-77]. They are inactive at resting membrane potentials but are activated when membrane potentials are depolarized, resulting in Ca^{2+} entry. Intracellular Ca^{2+} increase elicits contraction in muscles and activation of signaling pathways in neurons [77]. VDCCs were initially considered to be the main entry gate for Zn^{2+} from the extracellular space into cytoplasm [78, 79].

Zn^{2+} influx into cells was first described in invertebrates 35 years ago [80]. Shortly after that, VDCCs were found to be a route for Zn^{2+} entry as demonstrated by the inhibition of Zn^{2+} influx with VDCC blockers, such as verapamil [81]. Like invertebrates, VDCCs are also permeable to Zn^{2+} in mammalian neurons, cardiac myocytes, pancreatic β cells, and chromaffin cells [78, 81-85]. Variations in VDCC configuration and subunit composition likely affect the Zn^{2+} permeablity of these channels. Inhibition of Zn^{2+} influx by ω-conotoxin and nimodipine [82, 83, 86, 87], which block different types of VDCCs, suggests that the extent of Zn^{2+} permeability may be determined by the presence of specific subunits. Although VDCCs conduct Zn^{2+}, interestingly, they are also known to be blocked by Zn^{2+} [75-79]. For example, it has been demonstrated that Zn^{2+} blocks Ca^{2+} entry through the same channels [87]. In addition, extracellular acidic pH enhances Zn^{2+} entry through VDCCs and modifies the ratio of Zn^{2+}/Ca^{2+} flux [87], which may suggest that an increased Zn^{2+} influx contributes to neurotoxicity in conditions such as brain ischemia and lactic acidosis. Interestingly, recent reports have indicated that VDCCs located on intracellular endplasmic reticulum also contribute to zinc signaling and the downstream gene expression in mast cells [88].

Glutamate receptors

Glutamate is the major excitatory neurotransmitter in the central nervous system and is released into the synaptic cleft when stimulated by an action potential at the synaptic terminus. The binding of glutamate to its receptor on the postsynaptic membrane activates the receptor-gated cation channels. Glutamate receptors are divided into two major groups: ionotropic receptors, coupled to ion channels, and metabotropic receptors, coupled to G-protein mediated signaling pathways (mGluRs) [89-93]. The ionotropic receptors include N-methyl-D-aspartate receptors (NMDA-Rs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-Rs), and kainate receptors. When these receptors are activated by glutamate, influx of cations, such as Na^{+} and Ca^{2+}, is facilitated. Activation of glutamate receptor-gated channels is important for physiological functions such as neurotransmission. Zn^{2+} entry through NMDA-Rs has been shown to enhance field excitatory postsynaptic potentials [94]. Over activation of these channels are also known to contribute to neurotoxicity under a varitey of pathophysiological contions such as stroke and epilepsy [95].

NMDA-Rs were first reported to be blocked by Zn^{2+} [71, 72, 96]. Later, they were found to be Zn^{2+} -permeable although the permeablilty to Zn^{2+} is much lower than Ca^{2+} [97]. Physiological and pathophysiological events mediated by Zn^{2+} via NMDA-Rs seem to be dependent more on Zn^{2+} modulation of the channel rather than Zn^{2+} conductance [98, 99].

Early studies reported that Zn^{2+} can modulate the activity of AMPA-Rs by an unknown mechanism [71]. Weiss and colleagues later found that Zn^{2+} permeates AMPA-Rs, producing toxic effects [82]. They also demonstrated a clear relationship between Zn^{2+} toxicity and the activation of AMPA-Rs, which plays a role in ischemic neuronal injury [34]. Additional studies suggests that Zn^{2+} entry mediated by AMPA-Rs contributes to neurotoxicity more than VDCCs and NMDA-Rs [84, 100]. Interestingly, in neocortex, interneurons are more sensitive to AMPA-Rs mediated Zn^{2+} toxicity than other neurons [101]. Furthermore, Zn^{2+} inhibits gene expression of GluR2, a subunit of AMPA-Rs that regulates Ca^{2+} permeability; this inhibibition results in Ca^{2+} and Zn^{2+} toxicity [102, 103]. Unlike VDCCs, Ca^{2+} entry through AMPA-Rs are not influenced by Zn^{2+} [104].

Nicotinic acethylcoline receptors (nAch-Rs)

There are two types of acetylcholine receptors (Ach-R): ionotropic and metabotropic ones. Ionotropic Ach-Rs are also called nicotinic receptors (nAch-Rs). They are expressed mainly in muscle endoplate, at neuro-muscular junctions and throughout the nervous system in vertebrates [105, 106]. Neuronal nAch-Rs have a large variety of subunit components depending on their anatomical distribution. Their major role is to modulate neuronal function. In contrast, muscular nAch-Rs play an exlusive role in neuro-muscular transmission. Although neuronal nAch-Rs are in general highly Ca^{2+}-permeable, muscular ones are less permeable to Ca^{2+} [107]. Therefore, secondary activation of VDCCs follwing nAch-Rs-mediated depolarization is required for Ca^{2+} accumulation and muscle contraction. Regardless of its relatively low Ca^{2+} permeability, Zn^{2+} conductance has been reported for nAch-Rs found on endplates in muscles [108] and in adrenal chromaffin cells [109]. Similar to native nAch-Rs, Zn^{2+} permeability was demonstrated in cloned nAch-Rs [110]. It has been shown that γ- and ε-subuits are Zn^{2+} -permeable whereas other subunits are not [110]. The physiological and/or pathophysiolological sigificance of Zn^{2+} permeability in nAch-Rs, however, remains to be elucidated.

Transient Receptor Potential (Trp) channels

First member of Trp channels was discovered in *D. melanogaster*, where a genetic mutation of the gene induced an abnormal photosensitivity [111, 112]. The Trp superfamily of ion channels now includes nearly thirty genes [113], which are divided into 6 subfamilies [113, 114]. Sequcence homology is conserved between flies and higher organisms and members of the Trp family are cation-permeable. Trp channels have widespread distribution and play important roles in various physiological and pathophysiological conditions. For example, local thermal sensation is largely attributable to certain Trp channels and genetic mutations of some Trp channels elicit a variety of diseases such as epilepsy, leukemia and hypertension [113, 115]. Recent studies of cation permeation using electrophysiology or fluorescent Zn^{2+} imaging have demonstrated that several members of the Trp family are Zn^{2+} permeable.

TrpM6/7

TrpM7 was the first among Trp channels found to be Zn^{2+} permeable [116]. TrpM6/7 are unique among channels because they also possess a kinase domain [117, 118]. TrpM7 plays

a key role in intracellular Mg^{2+} homeostasis and in neuronal Ca^{2+} toxicity in ischemic conditions [118-122]. Monteilh-Zoller et al. demonstrated that the permeability of TrpM7 channels for Zn^{2+} ions is four-fold higher compared to Ca^{2+} [116]. TrpM7-overexpressing cells showed intracellular Zn^{2+} accumulation as indicated by increased fluorescence of FluoZin-3, a recently developed Zn^{2+} fluorescent indicator [123]. This Zn^{2+} accumulation contributes to, at least in part, functional gene expression in neuronal cells through the activation of metal-responsive element, which is important for monitoring intracellular metal levels [123-125]. Thus, not only because of abnormal cellular function (see above), but also because of changes in various gene expression, Zn^{2+} overload through TrpM7 will facilitate cytotoxicity [123, 126]. TrpM6, a homolog of TrpM7, also has a kinase domain. It has been implicated in Mg^{2+} homeostasis in the intestines and kidneys [120, 127]. Similar to TrpM7, TrpM6 has also been shown to be Zn^{2+} permeable [128].

TrpM3

TrpM3, a member of TRP channels highly expressed in brain and in pancreatic β cells [129, 130], is important for insulin release [131]. Zn^{2+} has been found in the vesicles of these cells, which is co-released with insulin and contributes to insulin function [131, 132]. While ZnT8 was speculated to facilitate Zn^{2+} uptake from cytoplasm into vesicles [133], Zn^{2+} permeability has recently been shown in one of the TrpM3 variants in native pancreatic β cells [134]. TrpM3 appears to contribute to Zn^{2+} permeation comparable to VDCCs [134]. Since activation of TrpM3 produces depolarization of β cells, which can lead to additional Zn^{2+} influx through VDCCs, further segregation of functional significance of Zn^{2+} entry between TrpM3 and VDCCs may be explored in the near future.

TrpA1

TrpA1 channels are highly expressed in dorsal root ganglia neurons [135, 136]. TrpA1 is stimulated by irritants such as mustard oil and noxious cold [135-139]. They are also expressed in the respiratory tract and play a role in hypersensitivity and inflammatory reactions associated with components of tobacco that activate the TrpA1 channels [140, 141]. Hu and colleagues demonstrated that TrpA1 channels act as a feedforward system which utilizes Zn^{2+} to further activate the channels [142]. TrpA1 deficient mice seem to feel less pain by Zn^{2+} injection whereas Zn^{2+} injection leads to apparent painful behavior in wild-type mice. This finding suggests that TrpA1 channels expressed in peripheral nerves are associated with pain sensation. Further studies showed that Asp 915 plays an important role in Zn^{2+} entry through TrpA1 while C641 and Cys 1021 are involved in the sensitivity of TrpA1 channels to intracellular Zn^{2+} activation [142].

TrpV6

TrpV6 is highly expressed in intestines, kidney and placenta [143-145]. Although TrpV6 is thought to contribute to Ca^{2+} absorption through its high Ca^{2+} conductance, the *in vivo* function of this channel still remains largely unknown. Some reports indicate that significantly higher TrpV6 expression is found on prostate cancer cells and its expression is associated with cancer progression [146-148]. Zn^{2+} also permeates TrpV6 channel [149], at least in TrpV6-overexpressing cells. Of note, TrpV5/6 in fishes are also known to transport

Zn^{2+} [150, 151] and showed a transient decrease of gene expression during Zn^{2+} excess [152], suggesting that permeability of Zn^{2+} might play a role in all vertebrates.

TrpC6

TrpC6 channels are expressed ubiquitously [153, 154]. It is known that Ca^{2+} is much more permeable than Na^{+} through these channels and that intra- and extracellular Ca^{2+} potentiates and inhibits the channel activity, respectively [154]. Because of its broad distribution, TrpC6 channels are suggested to play a significant role in various organ systems including kidney and brain [155, 156]. The Zn^{2+} permeability of these channels has recently been demonstrated in neurons and in transfected HEK293 cells [157]. Interestingly, TrpC6 has been suggested to be associated with Zn^{2+} accumulation in the nucleus. Zn^{2+} may also regulate TrpC6 channel activity by modifying the effect of Ca^{2+}. Although the physiological/pathophysiological significance of Zn^{2+} permeability for TrpC6 is still unclear, a recent report indicates that its overexpression facilitates Zn^{2+} accumulation in endo/lysosomes and upregulation of MTs [158]. However, considering that inhibition of TrpC6 degradation ameliorates ischemic brain injury [156], Zn^{2+} permeation through this channel does not seem to exacerbate or produce neurotoxicity.

TRPML1

TRPML1, also known as mucolipin-1, is localized to endosomes and lysosomes [159, 160]. Mutation of TRPML1 gene is associated with mucolipidosis, which is a rare autosomal recessive neurodegenerative disorder [161-163]. The clinical symptoms are severe with growth and psychomotor impairment in early stage of development (mostly 0 – 3 years). Most patients can not walk and speak over their lifetime, while half of the patients also have aberrant iron metabolism and anemia [164, 165]. Dong et al. discovered that TRPML1 is an iron-permeable channel and that changes in cellular iron level may play an important role in hereditary mucolipidosis [166]. They also found that TRPML1 conducts Zn^{2+} [166]. Recent findings have shown that knockdown of TRPML1 leads to abnormal cellular zinc localization and accumulation, for example, TRPML1 knockout mice have a significantly increased level of Zn^{2+} in brain tissues [167, 168]. These findings suggest that abnormal zinc homeostasis derived from TRPML1 mutation may contribute to neurological dysfunction.

Prospective

The activities of a number of channels are modulated by Zn^{2+} [169-171]. Addition of Zn^{2+} may enhance or inhibit the channel activity, resulting in increased or decreased current amplitude. For some channels, changes in current amplitude maybe mediated by Zn^{2+} permeation. As mentioned above, amino acids such as histidine, cysteine, glutamate and aspartate are likely to be preferable for Zn^{2+} coordination of catalytic activities and protein architecture [5, 22]. Amino acid substitution may therefore change the activity and function of channels that are modulated by Zn^{2+} [172-178]. However, one may also consider the possibility that certain amino acids are required for Zn^{2+} permeation and an examination of existing channels may facilitate the discovery of other Zn^{2+} -permeable channels. For

instance, Zn^{2+} permeability might be revealed in family members of Trp channels, most of which are found to permeate various divalent cations [113, 114].

Recent advancement in fluorescent Zn^{2+} indicators has made the investigation of intracellular Zn^{2+} dynamics much easier in comparison to previous probes. Historically, fluorescent Zn^{2+} indicators such as zinquin have been used for many decades [83, 179-184]. However, zinquin-like indicators are limited in specificity or have low affinity for Zn^{2+}. Some of them have high affinity for Ca^{2+} [51, 83, 185], or are influenced by Ca^{2+} [186-188]. In contrast, FluoZin-3, which was utilized to study Zn^{2+} permeation in TrpA1 and TrpM3, has a higher affinity (Kd = 3 ~ 15 nM) for Zn^{2+}, which is close to the intracellular Zn^{2+} concentration in resting conditions [11, 189, 190]. Of special note, FluoZin-3 is insensitive to Ca^{2+}, which has a significant advantage over previous indicators that cannot differentiate between Zn^{2+} and Ca^{2+} signals. With the emergence of new indicators, examinations of Zn^{2+} and Ca^{2+}, simultaneously, using different fluorescent indicators have been reported [191, 192]. These advancements are likely to provide a better understanding of Zn^{2+} regulation, conductance and functional importance.

Acknowledgements

This work was supported by NIH R01NS66027, NIMHD S21-MD000101, U54NS083932, AHA 0840132N, and ALZ IIRG-10-173350.

References

1. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev. 1993; 73:79–118. [PubMed: 8419966]
2. Beyersmann D, Haase H. Functions of zinc in signaling, proliferation and differentiation of mammalian cells. Biometals. 2001; 14:331–341. [PubMed: 11831463]
3. Berg JM, Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. Science. 1996; 271:1081–1085. [PubMed: 8599083]
4. McCall KA, Huang C, Fierke CA. Function and mechanism of zinc metalloenzymes. J Nutr. 2000; 130:1437S–1446S. [PubMed: 10801957]
5. Patel K, Kumar A, Durani S. Analysis of the structural consensus of the zinc coordination centers of metalloprotein structures. Biochim Biophys Acta. 2007; 1774:1247–1253. [PubMed: 17855175]
6. Andreini C, Banci L, Bertini I, Rosato A. Zinc through the three domains of life. J Proteome Res. 2006; 5:3173–3178. [PubMed: 17081069]
7. Sandstead HH. Zinc deficiency. A public health problem? Am J Dis Child. 1991; 145:853–859. [PubMed: 1858720]
8. Fischer Walker C, Black RE. Zinc and the risk for infectious disease. Annu Rev Nutr. 2004; 24:255–275. [PubMed: 15189121]
9. Skouby AP. Vascular lesions in diabetics with a special reference to the influence of treatment. Acta Med Scand Suppl. 1956; 317:1–46. [PubMed: 13372203]
10. Mobasheri A, Avila J, Cozar-Castellano I, Brownleader MD, Trevan M, Francis MJ, Lamb JF, Martin-Vasallo P. Na^+, K^+-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. Biosci Rep. 2000; 20:51–91. [PubMed: 10965965]
11. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. Nat Rev Neurosci. 2005; 6:449–462. [PubMed: 15891778]
12. Colvin RA, Bush AI, Volitakis I, Fontaine CP, Thomas D, Kikuchi K, Holmes WR. Insights into Zn^{2+} homeostasis in neurons from experimental and modeling studies. Am J Physiol Cell Physiol. 2008; 294:C726–742. [PubMed: 18184873]

13. Haase, H.; Rink, L. The physiological role of zinc ions in mammalian signal transduction. Nova Science Publishers, Inc.; 2007.
14. Choi DW. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci. 1988; 11:465–469. [PubMed: 2469166]
15. Berridge MJ. Neuronal calcium signaling. Neuron. 1998; 21:13–26. [PubMed: 9697848]
16. Sherwood, L. Human physiology: from cells to systems. 7th ed.. CENGAGE Learning; Belmont, CA, USA: 2010.
17. Fleischer, M. Natural sources and distribution of zinc.. In: Henkin, RI., editor. Zinc. Univ Park Press; Baltimore, MA, USA: 1979. p. 19-29.
18. Smith, WF. Principels of material science and engineering. 2nd ed.. Mc Graw-Hill Companies; Mishawaka, IN, USA: 1990.
19. B. JW. The place of zinc, cadmium, and mercury in the periodic table. J Chem Educ. 2003; 80:952–961.
20. Butler A. Acquisition and utilization of transition metal ions by marine organisms. Science. 1998; 281:207–209. [PubMed: 9660742]
21. Pearson RG. Hard and soft acids and bases. J Am Chem Soc. 1963; 85:3533–3539.
22. Gregory DS, Martin AC, Cheetham JC, Rees AR. The prediction and characterization of metal binding sites in proteins. Protein Eng. 1993; 6:29–35. [PubMed: 8433968]
23. Todd WR, Elvehjem CA, Hart EB. Zinc in the nutrition of the rat. Am J Physiol. 1933; 107:146–156.
24. Raurlin J. Etudes cliniques sur lar vegetation. Ann Sci Nat Bot Biol Veg. 1869; 11:93.
25. Sandstead, HH. Zinc and human nutrition.. In: Bronner, F.; Coburn, JW., editors. Disorders of mineral and metabolism: Trace minerals Deep River. Jonathan Grobe Books; IA, USA: 1981. p. 93-157.
26. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med. 1961; 31:532–546. [PubMed: 14488490]
27. Cunnane, SC. Zinc: Clinincal and Biochemical Significance. CRC Press; Boca Raton, FL: 1988.
28. Hamidge, KM.; Casey, CE.; Krebs, NF. Trace Elements in Human and Animal Nutrition. 5 edition ed.. Academic Press; Orlando, FL: 1988.
29. Heyneman CA. Zinc deficiency and taste disorders. Annals of Pharmacotherapy. 1996; 30:186–187. [PubMed: 8835055]
30. Apgar J. Zinc and reproduction. Annu Rev Nutr. 1985; 5:43–68. [PubMed: 3896272]
31. Moynanan EJ. Acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. Lancet. 1974; 17:399–400.
32. Kury S, Dreno B, Bezieau S, Giraudet S, Kharfi M, Kamoun R, Moisan JP. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. Nat Genet. 2002; 31:239–240. [PubMed: 12068297]
33. Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. Am J Hum Genet. 2002; 71:66–73. [PubMed: 12032886]
34. Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY, Choi DW. The role of zinc in selective neuronal death after transient global cerebral ischemia. Science. 1996; 272:1013–1016. [PubMed: 8638123]
35. Suh SW, Chen JW, Motamedi M, Bell B, Listiak K, Pons NF, Danscher G, Frederickson CJ. Evidence that synaptically-released zinc contributes to neuronal injury after traumatic brain injury. Brain Res. 2000; 852:268–273. [PubMed: 10678752]
36. Capasso M, Jeng JM, Malavolta M, Mocchegiani E, Sensi SL. Zinc dyshomeostasis: a key modulator of neuronal injury. J Alzheimers Dis. 2005; 8:93–108. [PubMed: 16308478]
37. Bossy-Wetzel E, Talantova MV, Lee WD, Scholzke MN, Harrop A, Mathews E, Gotz T, Han J, Ellisman MH, Perkins GA, Lipton SA. Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K^+ channels. Neuron. 2004; 41:351–365. [PubMed: 14766175]
38. Sensi SL, Paoletti P, Bush AI, Sekler I. Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci. 2009; 10:780–791. [PubMed: 19826435]

39. Shahbaz AU, Zhao T, Zhao W, Johnson PL, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Calcium and zinc dyshomeostasis during isoproterenol-induced acute stressor state. Am J Physiol Heart Circ Physiol. 2011; 300:H636–H644. [PubMed: 21076021]
40. Lee JM, Kim YJ, Ra H, Kang SJ, Han S, Koh JY, Kim YH. The involvement of caspase-11 in TPEN-induced apoptosis. FEBS Lett. 2008; 582:1871–1876. [PubMed: 18474237]
41. Cooper RG. Zinc toxicology following particulate inhalation. Indian J Occup Environ Med. 2008; 12:10–13. [PubMed: 20040991]
42. Salzman MB, Smith EM, Koo C. Excessive oral zinc supplementation. J Pediatr Hematol Oncol. 2002; 24:582–584. [PubMed: 12368702]
43. Grissinger M. A fatal zinc overdose in a neonate: confusion of micrograms with milligrams. PT. 2011; 36:393–407.
44. Prasad AS, Miale A Jr. Farid Z, Sandstead HH, Schulert AR. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypognadism. J Lab Clin Med. 1963; 61:537–549. [PubMed: 13985937]
45. Palm R, Sjostrom R, Hallmans G. Optimized atomic absorption spectrophotometry of zinc in cerebrospinal fluid. Clin Chem. 1983; 29:486–491. [PubMed: 6825259]
46. Molina JA, Jimenez-Jimenez FJ, Aguilar MV, Meseguer I, Mateos-Vega CJ, Gonzalez-Munoz MJ, de Bustos F, Porta J, Orti-Pareja M, Zurdo M, Barrios E, Martinez-Para MC. Cerebrospinal fluid levels of transition metals in patients with Alzheimer's disease. J Neural Transm. 1998; 105:479–488. [PubMed: 9720975]
47. Bodgen JD, Troiano RA, Joselow MM. Copper, zinc magnesium, and calcium in plasma and cerebrospinal fluid of patients with neurological diseases. Clin Chem. 1977; 23:485–489. [PubMed: 837537]
48. Perez-Clausell J, Danscher G. Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. Brain Res. 1985; 337:91–98.
49. Slomianka L, Geneser FA. Postnatal development of zinc-containing cells and neuropil in the hippocampal region of the mouse. Hippocampus. 1997; 7:321–340. [PubMed: 9228529]
50. Pierrel F, Cobine PA, Winge DR. Metal Ion availability in mitochondria. Biometals. 2007; 20:675–682. [PubMed: 17225062]
51. Ohana E, Segal D, Palty R, Dien TT, Moran A, Sensi SL, Weiss JH, Hershfinkel M, Sekler I. A sodium zinc exchange mechanism is mediating extrusion of zinc in mammalian cells. J Biol Chem. 2004; 279:4278–4284. [PubMed: 14581475]
52. Simons TJ. Calcium-dependent zinc efflux in human red blood cells. J Membr Biol. 1991; 123:73–82. [PubMed: 1774776]
53. Wang J, Cooper MD. Histidine residue in the zinc-binding motif of aminopeptidase A is critical for enzymatic activity. Proc Natl Acad Sci U S A. 1993; 90:1222–1226. [PubMed: 8433982]
54. Schauder B, Schomburg L, Kohrle J, Bauer K. Cloning of a Cdna-Encoding an Ectoenzyme That Degrades Thyrotropin-Releasing-Hormone. Proc Natl Acad Sci U S A. 1994; 91:9534–9538. [PubMed: 7937801]
55. Zielinska W, Barata H, Chini EN. Metabolism of cyclic ADP-ribose: Zinc is an endogenous modulator of the cyclase/NAD glycohydrolase ratio of a CD38-like enzyme from human seminal fluid. Life Sci. 2004; 74:1781–1790. [PubMed: 14741735]
56. Davies PA, Wang W, Hales TG, Kirkness EF. A novel class of ligand-gated ion channel is activated by Zn^{2+}. J Biol Chem. 2003; 278:712–717. [PubMed: 12381728]
57. Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, Schwartz TW. GPR39 signaling is stimulated by zinc ions but not by obestatin. Endocrinology. 2007; 148:13–20. [PubMed: 16959833]
58. Yasuda S, Miyazaki T, Munechika K, Yamashita M, Ikeda Y, Kamizono A. Isolation of Zn^{2+} as an endogenous agonist of GPR39 from fetal bovine serum. J Recept Signal Transduct Res. 2007; 27:235–246. [PubMed: 17885920]
59. West AK, Hidalgo J, Eddins D, Levin ED, Aschner M. Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. Neurotoxicology. 2008; 29:489–503. [PubMed: 18313142]

60. Andrews GK. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol. 2000; 59:95–104. [PubMed: 10605938]
61. Palmiter RD, Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J. 1995; 14:639–649. [PubMed: 7882967]
62. Palmiter RD, Cole TB, Quaife CJ, Findley SD. ZnT-3, a putative transporter of zinc into synaptic vesicles. Proc Natl Acad Sci U S A. 1996; 93:14934–14939. [PubMed: 8962159]
63. Kambe T. An overview of a wide range of functions of ZnT and Zip zinc transporters in the secretory pathway. Biosci Biotechnol Biochem. 2011; 75:1036–1043. [PubMed: 21670538]
64. Liuzzi JP, Cousins RJ. Mammalian zinc transporters. Annu Rev Nutr. 2004; 24:151–172. [PubMed: 15189117]
65. Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr. 2009; 29:153–176. [PubMed: 19400752]
66. Eide DJ. Zinc transporters and the cellular trafficking of zinc. Biochim Biophy Acta. 2006; 1763:711–722.
67. Keilin D, Mann T. Carbonic anhydrase. Purification and nature of the enzyme. Biochem J. 1940; 34:1163–1176. [PubMed: 16747299]
68. Keilin D, Mann T. Carbonic Anhydrase. Nature. 1939; 144:442–443.
69. Duce JA, Tsatsanis A, Cater MA, James SA, Robb E, Wikhe K, Leong SL, Perez K, Johanssen T, Greenough MA, Cho HH, Galatis D, Moir RD, Masters CL, McLean C, Tanzi RE, Cappai R, Barnham KJ, Ciccotosto GD, Rogers JT, Bush AI. Iron-Export Ferroxidase Activity of beta-Amyloid Precursor Protein Is Inhibited by Zinc in Alzheimer's Disease. Cell. 2010; 142:857–867. [PubMed: 20817278]
70. Draguhn A, Verdorn TA, Ewert M, Seeburg PH, Sakmann B. Functional and mlecular disfunction between recombinant rat GABAA receptor subtypes by Zn^{2+}. Neuron. 1990; 5:781–788. [PubMed: 1702644]
71. Peters S, Koh J, Choi DW. Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. Science. 1987; 236:589–593. [PubMed: 2883728]
72. Westbrook GL, Mayer ML. Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. Nature. 1987; 328:640–643. [PubMed: 3039375]
73. Lee JY, Cole TB, Palmiter RD, Koh JY. Accumulation of zinc in degenerating hippocampal neurons of ZnT3-null mice after seizures: evidence against synaptic vesicle origin. J Neurosci. 2000; 20:RC79. [PubMed: 10807937]
74. Kodirov SA, Takizawa S, Joseph J, Kandel ER, Shumyatsky GP, Bolshakov VY. Synaptically released zinc gates long-term potentiation in fear conditioning pathways. Proc Natl Acad Sci U S A. 2006; 103:15218–15223. [PubMed: 17005717]
75. Tsien RW. calcium channels in excitable cell membranes. Ann Rev Physiol. 1983; 45:341–358. [PubMed: 6303205]
76. Yamakage M, Namiki A. Calcium channels -basic aspects of their structure, function and gene encoding; anesthetic action on the channels -a review. Can J Anaesth. 2002; 49:151–164. [PubMed: 11823393]
77. Catterall WA. Voltage-gated calcium channels. Cold Spring Harb Perspect Biol. 2011; 3:a003947. [PubMed: 21746798]
78. Gyulkhandanyan AV, Lee SC, Bikopoulos G, Dai F, Wheeler MB. The Zn^{2+}-transporting pathways in pancreatic beta-cells: a role for the L-type voltage-gated $Ca2^{+}$ channel. J Biol Chem. 2006; 281:9361–9372. [PubMed: 16407176]
79. Priel T, Hershfinkel M. Zinc influx and physiological consequences in the beta-insulinoma cell line, Min6. Biochem Biophys Res Commun. 2006; 346:205–212. [PubMed: 16750816]
80. Fukuda J, Kawa K. Permeation of manganese, cadmium, zinc, and beryllium through calcium channels of an insect muscle membrane. Science. 1977; 196:309–311. [PubMed: 847472]
81. Kawa K. Zinc-dependent action potentials in giant neurons of the snail, Euhadra quaestia. J Membr Biol. 1979; 49:325–344. [PubMed: 480340]
82. Weiss JH, Hartley DM, Koh JY, Choi DW. AMPA receptor activation potentiates zinc neurotoxicity. Neuron. 1993; 10:43–49. [PubMed: 7678965]

83. Sensi SL, Canzoniero LM, Yu SP, Ying HS, Koh JY, Kerchner GA, Choi DW. Measurement of intracellular free zinc in living cortical neurons: routes of entry. J Neurosci. 1997; 17:9554–9564. [PubMed: 9391010]
84. Sensi SL, Yin HZ, Carriedo SG, Rao SS, Weiss JH. Preferential Zn^{2+} influx through Ca^{2+}-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production. Proc Natl Acad Sci U S A. 1999; 96:2414–2419. [PubMed: 10051656]
85. Atar D, Backx PH, Appel MM, Gao WD, Marban E. Excitation-transcription coupling mediated by zinc influx through voltage-dependent calcium channels. J Biol Chem. 1995; 270:2473–2477. [PubMed: 7852308]
86. Striessnig J, Koschak A. Exploring the function and pharmacotherapeutic potential of voltage-gated Ca^{2+} channels with gene Knockout models. Channels. 2008; 2:233–251. [PubMed: 18719397]
87. Kerchner GA, Canzoniero LMT, Yu SP, Ling C, Choi DW. Zn^{2+} current is mediated by voltage-gated Ca^{2+} channels and enhanced by extracellular acidity in mouse cortical neurones. J Physiol. 2000; 528:39–52. [PubMed: 11018104]
88. Yamasaki S, Hasegawa A, Hojyo S, Ohashi W, Fukada T, Nishida K, Hirano T. A novel role of the L-type calcium channel alpha1D subunit as a gatekeeper for intracellular zinc signaling: zinc wave. PLoS One. 2012; 7:e39654. [PubMed: 22745805]
89. 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. Pharmacol Rev. 2010; 62:405–496. [PubMed: 20716669]
90. Dhami GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. Pharmacol Ther. 2006; 111:260–271. [PubMed: 16574233]
91. Rousseaux CG. A review of glutamate receptors I: current understanding of their biology. J Toxicol Pathol. 2008; 21:25–51.
92. Kew JN, Kemp JA. Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl). 2005; 179:4–29. [PubMed: 15731895]
93. Li MH, Inoue K, Si HF, Xiong ZG. Calcium-permeable ion channels involved in glutamate receptor-independent ischemic brain injury. Acta Pharmacol Sin. 2011; 32:734–740. [PubMed: 21552295]
94. Kim TY, Hwang JJ, Yun SH, Jung MW, Koh JY. Augmentation by zinc of NMDA receptor-mediated synaptic responses in CA1 of rat hippocampal slices: mediation by Src family tyrosine kinases. Synapse. 2002; 46:49–56. [PubMed: 12211081]
95. Lau A, Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. Pflugers Arch. 2010; 460:525–542. [PubMed: 20229265]
96. Hori N, Galeno T, Carpenter DO. Responses of pyriform cortex neurons to excitatory amino acids: voltage dependence, conductance changes, and effects of divalent cations. Cell Mol Neurobiol. 1987; 7:73–90. [PubMed: 3297341]
97. Koh JY, Choi DW. Zinc toxicity on cultured cortical-neurons - involvement of N-methyl-D-aspartate receptors. Neuroscience. 1994; 60:1049–1057. [PubMed: 7936205]
98. Nozaki C, Vergnano AM, Filliol D, Ouagazzal AM, Le Goff A, Carvalho S, Reiss D, Gaveriaux-Ruff C, Neyton J, Paoletti P, Kieffer BL. Zinc alleviates pain through high-affinity binding to the NMDA receptor NR2A subunit. Nat Neurosci. 2011; 14:1017–1022. [PubMed: 21725314]
99. Fayyazuddin A, Villarroel A, Le Goff A, Lerma J, Neyton J. Four residues of the extracellular N-terminal domain of the NR2A subunit control high-affinity Zn^{2+} binding to NMDA receptors. Neuron. 2000; 25:683–694. [PubMed: 10774735]
100. Weiss JH, Sensi SL. Ca^{2+}-Zn^{2+} permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration. Trends Neurosci. 2000; 23:365–371. [PubMed: 10906800]
101. Martinez-Galan JR, Diaz C, Juiz JM. Histochemical localization of neurons with zinc-permeable AMPA/kainate channels in rat brain slices. Brain Res. 2003; 963:156–164. [PubMed: 12560121]
102. Calderone A, Jover T, Mashiko T, Noh K, Tanaka H, Bennett MVL, Zukin RS. Late calcium EDTA rescues hippocampal CA1 neurons from global ischemia-induced death. J Neurosci. 2004; 24:9903–9913. [PubMed: 15525775]

103. Pellegrini-Giampietro DE, Gorter JA, Bennett MVL, Zukin RS. The GluR2 (GluR-B) hypothesis: Ca^{2+}-permeable AMPA receptors in neurological disorders. Trends Neurosci. 1997; 20:464–470. [PubMed: 9347614]
104. Jia Y, Jeng JM, Sensi SL, Weiss JH. Zn^{2+} currents are mediated by calcium-permeable AMPA/kainate channels in cultured murine hippocampal neurones. J Physiol. 2002; 543:35–48. [PubMed: 12181280]
105. Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: new perspectives from knockout mice. Trends Pharmacol Sci. 2000; 21:211–217. [PubMed: 10838608]
106. Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. Heterogeneity and complexity of native brain nicotinic receptors. Biochem Pharmacol. 2007; 74:1102–1111. [PubMed: 17597586]
107. Lindstrom JM. Nicotinic acetylcholine receptors of muscles and nerves: comparison of their structures, functional roles, and vulnerability to pathology. Ann N Y Acad Sci. 2003; 998:41–52. [PubMed: 14592862]
108. Adams DJ, Dwyer TM, Hille B. The permeability of endplate channels to monovalent and divalent metal cations. J Gen Physiol. 1980; 75:493–510. [PubMed: 6247423]
109. Vega MT, Villalobos C, Garrido B, Gandia L, Bulbena O, Garciasancho J, Garcia AG, Artalejo AR. Permeation by Zinc of Bovine Chromaffin Cell Calcium Channels - Relevance to Secretion. Pflug Arch Eur J Phy. 1994; 429:231–239.
110. Ragozzino D, Giovannelli A, Degasperi 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. [PubMed: 11080253]
111. Mikne B. Drosophila mutant with a transducer defect. Biophys Struct Mech. 1977; 3:59–64. [PubMed: 870103]
112. Montell C, Jones K, Hafen E, Rubin G. Rescue of the Drosophila phototransduction mutation trp by germline transformation. Science. 1985; 230:1040–1043. [PubMed: 3933112]
113. Clapham DE. TRP channels as cellular sensors. Nature. 2003; 426:517–524. [PubMed: 14654832]
114. Padinjat R, Andrews S. TRP channels at a glance. J Cell Sci. 2004; 117:5707–5709. [PubMed: 15537828]
115. Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. Physiol Rev. 2007; 87:165–217. [PubMed: 17237345]
116. Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, Fleig A. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. J Gen Physiol. 2003; 121:49–60. [PubMed: 12508053]
117. Runnels LW, Yue L, Clapham DE. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science. 2001; 291:1043–1047. [PubMed: 11161216]
118. Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A. LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. Nature. 2001; 411:590–595. [PubMed: 11385574]
119. Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurosaki T, Fleig A, Scharenberg AM. Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. Cell. 2003; 114:191–200. [PubMed: 12887921]
120. Schlingmann KP, Weber S, Peters M, Nejsum LN, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. Nat Genet. 2002; 31:166–170. [PubMed: 12032568]
121. Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. Nat Genet. 2002; 31:171–174. [PubMed: 12032570]
122. Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonald JF, Tymianski M. A key role for TRPM7 channels in anoxic neuronal death. Cell. 2003; 115:863–877. [PubMed: 14697204]

123. Inoue K, Branigan D, Xiong ZG. Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. J Biol Chem. 2010; 285:7430–7439. [PubMed: 20048154]

124. LaRochelle O, Gagne V, Charron J, Soh JW, Seguin C. Phosphorylation is involved in the activation of metal-regulatory transcription factor 1 in response to metal ions. J Biol Chem. 2001; 276:41879–41888. [PubMed: 11551972]

125. Laity JH, Andrews GK. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). Arch Biochem Biophys. 2007; 463:201–210. [PubMed: 17462582]

126. Leng TD, Lin J, Sun HW, Zeng Z, O'Bryant Z, Inoue K, Xiong ZG. Local Anesthetic Lidocaine Inhibits TRPM7 Current and TRPM7-Mediated Zinc Toxicity. CNS Neurosci Ther. 2015; 21:32–39. [PubMed: 25169754]

127. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG. Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci U S A. 2012; 109:11324–11329. [PubMed: 22733750]

128. Li M, Jiang J, Yue L. Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7. J Gen Physiol. 2006; 127:525–537. [PubMed: 16636202]

129. Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J. Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. Nat Cell Biol. 2008; 10:1421–1430. [PubMed: 18978782]

130. Oberwinkler J, Lis A, Giehl KM, Flockerzi V, Philipp SE. Alternative splicing switches the divalent cation selectivity of TRPM3 channels. J Biol Chem. 2005; 280:22540–22548. [PubMed: 15824111]

131. Michael DJ, Ritzel RA, Haataja L, Chow RH. Pancreatic beta-cells secrete insulin in fast- and slow-release forms. Diabetes. 2006; 55:600–607. [PubMed: 16505221]

132. Dodson G, Steiner D. The role of assembly in insulin's biosynthesis. Curr Opin Struct Biol. 1998; 8:189–194. [PubMed: 9631292]

133. Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkhandanyan AV, Koshkin V, Tarasov AI, Carzaniga R, Kronenberger K, Taneja TK, da Silva Xavier G, Libert S, Froguel P, Scharfmann R, Stetsyuk V, Ravassard P, Parker H, Gribble FM, Reimann F, Sladek R, Hughes SJ, Johnson PR, Masseboeuf M, Burcelin R, Baldwin SA, Liu M, Lara-Lemus R, Arvan P, Schuit FC, Wheeler MB, Chimienti F, Rutter GA. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes. 2009; 58:2070–2083. [PubMed: 19542200]

134. Wagner TF, Drews A, Loch S, Mohr F, Philipp SE, Lambert S, Oberwinkler J. TRPM3 channels provide a regulated influx pathway for zinc in pancreatic beta cells. Pflugers Arch. 2010; 460:755–765. [PubMed: 20401728]

135. Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature. 2004; 427:260–265. [PubMed: 14712238]

136. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell. 2003; 112:819–829. [PubMed: 12654248]

137. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron. 2004; 41:849–857. [PubMed: 15046718]

138. Kosugi M, Nakatsuka T, Fujita T, Kuroda Y, Kumamoto E. Activation of TRPA1 channel facilitates excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord. J Neurosci. 2007; 27:4443–4451. [PubMed: 17442829]

139. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell. 2006; 124:1269–1282. [PubMed: 16564016]

140. Bessac BF, Sivula M, Von Hehn CA, Escalera J, Cohn L, Jordt SE. TRPA1 is a major oxidant sensor in murine airway sensory neurons. J Clin Invest. 2008; 118:1899–1910. [PubMed: 18398506]
141. Lee LY. TRPA1 ion channels: a gateway to airway irritation and reflex responses induced by inhaled oxidants. Journal of Physiology-London. 2010; 588:747–748.
142. Hu HZ, Bandell M, Petrus MJ, Zhu MX, Patapoutian A. Zinc activates damage-sensing TRPA1 ion channels. Nature Chemical Biology. 2009; 5:183–190.
143. Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM, Hediger MA. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J Biol Chem. 1999; 274:22739–22746. [PubMed: 10428857]
144. Moreau R, Daoud G, Bematchez R, Simoneau L, Masse A, Lafond J. Calcium uptake and calcium transporter expression by trophoblast cells from human term placenta. Biochimica Et Biophysica Acta-Biomembranes. 2002; 1564:325–332.
145. Peng JB, Brown EM, Hediger MA. Structural conservation of the genes encoding CaT1, CaT2, and related cation channels. Genomics. 2001; 76:99–109. [PubMed: 11549322]
146. Fixemer T, Wissenbach U, Flockerzi V, Bonkhoff H. Expression of the Ca^{2+}-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression. Oncogene. 2003; 22:7858–7861. [PubMed: 14586412]
147. Lehen'kyi V, Flourakis M, Skryma R, Prevarskaya N. TRPV6 channel controls prostate cancer cell proliferation via Ca^{2+}/NFAT-dependent pathways. Oncogene. 2007; 26:7380–7385. [PubMed: 17533368]
148. Lehen'kyi V, Raphael M, Oulidi A, Flourakis M, Khalimonchyk S, Kondratskyi A, Gordienko DV, Mauroy B, Bonnal JL, Skryma R, Prevarskaya N. TRPV6 determines the effect of vitamin D3 on prostate cancer cell growth. PLoS One. 2011; 6:e16856. [PubMed: 21347289]
149. Kovacs G, Tamas D, Bergeron MJ, Bernadett B, Suzuki Y, Akos Z, Hediger MA. Heavy metal cations permeate the TRPV6 epithelial cation channel. Cell Calcium. 2011; 49:43–55. [PubMed: 21146870]
150. Qiu A, Hogstrand C. Functional characterisation and genomic analysis of an epithelial calcium channel (ECaC) from pufferfish, Fugu rubripes. Gene. 2004; 342:113–123. [PubMed: 15527971]
151. Hogstrand C, Verbost PM, Bonga SE, Wood CM. Mechanisms of zinc uptake in gills of freshwater rainbow trout: interplay with calcium transport. Am J Physiol. 1996; 270:R1141–1147. [PubMed: 8928918]
152. Zheng D, Feeney GP, Handy RD, Hogstrand C, Kille P. Uptake epithelia behave in a cell-centric and not systems homeostatic manner in response to zinc depletion and supplementation. Metallomics. 2014; 6:154–165. [PubMed: 24301558]
153. Inoue R, Jensen LJ, Shi J, Morita H, Nishida M, Honda A, Ito Y. Transient receptor potential channels in cardiovascular function and disease. Circ Res. 2006; 99:119–131. [PubMed: 16857972]
154. Dietrich A, Gudermann T. TRPC6. Handb Exp Pharmacol. 2007:1251–1241.
155. Heeringa SF, Moller CC, Du J, Yue L, Hinkes B, Chernin G, Vlangos CN, Hoyer PF, Reiser J, Hildebrandt F. A novel TRPC6 mutation that causes childhood FSGS. PLoS One. 2009; 4:e7771. [PubMed: 19936226]
156. Du W, Huang J, Yao H, Zhou K, Duan B, Wang Y. Inhibition of TRPC6 degradation suppresses ischemic brain damage in rats. J Clin Invest. 2010; 120:3480–3492. [PubMed: 20811149]
157. Gibon J, Tu P, Bohic S, Richaud P, Arnaud J, Zhu MK, Boulay G, Bouron A. The over-expression of TRPC6 channels in HEK-293 cells favours the intracellular accumulation of zinc. Biochim Biophys Acta. 2011; 1808:2807–2818. [PubMed: 21864503]
158. Chevallet M, Jarvis L, Harel A, Luche S, Degot S, Chapuis V, Boulay G, Rabilloud T, Bouron A. Functional consequences of the over-expression of TRPC6 channels in HEK cells: impact on the homeostasis of zinc. Metallomics. 2014; 6:1269–1276. [PubMed: 24733507]
159. Manzoni M, Monti E, Bresciani R, Bozzato A, Barlati S, Bassi MT, Borsani G. Overexpression of wild-type and mutant mucolipin proteins in mammalian cells: effects on the late endocytic compartment organization. FEBS Lett. 2004; 567:219–224. [PubMed: 15178326]

160. Kiselyov K, Chen J, Rbaibi Y, Oberdick D, Tjon-Kon-Sang S, Shcheynikov N, Muallem S, Soyombo A. TRP-ML1 is a lysosomal monovalent cation channel that undergoes proteolytic cleavage. J Biol Chem. 2005; 280:43218–43223. [PubMed: 16257972]

161. Bargal R, Avidan N, Ben-Asher E, Olender Z, Zeigler M, Frumkin A, Raas-Rothschild A, Glusman G, Lancet D, Bach G. Identification of the gene causing mucolipidosis type IV. Nat Genet. 2000; 26:118–123. [PubMed: 10973263]

162. Bassi MT, Manzoni M, Monti E, Pizzo MT, Ballabio A, Borsani G. Cloning of the gene encoding a novel integral membrane protein, mucolipidin-and identification of the two major founder mutations causing mucolipidosis type IV. Am J Human Genet. 2000; 67:1110–1120. [PubMed: 11013137]

163. Sun M, Goldin E, Stahl S, Falardeau JL, Kennedy JC, Acierno JS Jr. Bove C, Kaneski CR, Nagle J, Bromley MC, Colman M, Schiffmann R, Slaugenhaupt SA. Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. Hum Mol Genet. 2000; 9:2471–2478. [PubMed: 11030752]

164. Schiffmann R, Dwyer NK, Lubensky IA, Tsokos M, Sutliff VE, Latimer JS, Frei KP, Brady RO, Barton NW, Blanchette-Mackie EJ, Goldin E. Constitutive achlorhydria in mucolipidosis type IV. Proc Natl Acad Sci U S A. 1998; 95:1207–1212. [PubMed: 9448310]

165. Bach G. Mucolipidosis type IV. Mol Genet Metab. 2001; 73:197–203. [PubMed: 11461186]

166. Dong XP, Cheng X, Mills E, Delling M, Wang F, Kurz T, Xu H. The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. Nature. 2008; 455:992–9926. [PubMed: 18794901]

167. Eichelsdoerfer JL, Evans JA, Slaugenhaupt SA, Cuajungco MP. Zinc dyshomeostasis is linked with the loss of mucolipidosis IV-associated TRPML1 ion channel. J Biol Chem. 2010; 285:34304–34308. [PubMed: 20864526]

168. Kukic I, Lee JK, Coblentz J, Kelleher SL, Kiselyov K. Zinc-dependent lysosomal enlargement in TRPML1-deficient cells involves MTF-1 transcription factor and ZnT4 (Slc30a4) transporter. Biochem J. 2013; 451:155–163. [PubMed: 23368743]

169. Harrison NL, Gibbons SJ. Zn^{2+}: an endogenous modulator of ligand- and voltage-gated ion channels. Neuropharmacology. 1994; 33:935–952. [PubMed: 7845550]

170. Elinder F, Arhem P. Metal ion effects on ion channel gating. Q Rev Biophysics. 2003; 36:373–427.

171. Smart TG, Hosie AM, Miller PS. Zn^{2+} ions: Modulators of excitatory and inhibitory synaptic activity. Neuroscientist. 2004; 10:432–442. [PubMed: 15359010]

172. Dunne EL, Hosie AM, Wooltorton JRA, Duguid IC, Harvey K, Moss SJ, Harvey RJ, Smart TG. An N-terminal histidine regulates Zn^{2+} inhibition on the murine GABAA receptor beta 3 subunit. Br J Pharmacol. 2002; 137:29–38. [PubMed: 12183328]

173. Horenstein J, Akabas MH. Location of a high affinity Zn^{2+} binding site in the channel of alpha 1 beta 1 gamma-aminobutyric $acid_A$ receptors. Mol Pharmacol. 1998; 53:870–877. [PubMed: 9584213]

174. Fisher JL, Macdonald RL. The role of an alpha subtype M_2-M_3 his in regulating inhibition of $GABA_A$ receptor current by zinc and other divalent cations. J Neurosci. 1998; 18:2944–2953. [PubMed: 9526011]

175. Wang TL, Hackam A, Guggino WB, Cutting GR. A single histidine residue is essential for zinc inhibition of GABA Rho-1 receptors. J Neurosci. 1995; 15:7684–7691. [PubMed: 7472519]

176. De Biasi M, Drewe JA, Kirsch GE, Brown AM. Histidine substitution identifies a surface position and confers Cs^+ selectivity on a K^+ pore. Biophys J. 1993; 65:1235–1242. [PubMed: 8241404]

177. Jiang Q, Inoue K, Wu X, Papasian CJ, Wang JQ, Xiong ZG, Chu XP. Cysteine 149 in the extracellular finger domain of acid-sensing ion channel 1b subunit is critical for zinc-mediated inhibition. Neuroscience. 2011; 193:89–99. [PubMed: 21767613]

178. Kurz LL, Klink H, Jakob I, Kuchenbecker M, Benz S, Lehmann-Horn F, Rudel R. Identification of three cysteines as targets for the Zn^{2+} blockade of the human skeletal muscle chloride channel. J Biol Chem. 1999; 274:11687–11692. [PubMed: 10206982]

179. Frederickson CJ, Kasarskis EJ, Ringo D, Frederickson RE. A quinoline fluorescence method for visualizing and assaying the histochemically reactive zinc (bouton zinc) in the brain. J Neurosci Methods. 1987; 20:91–103. [PubMed: 3600033]
180. Canzoniero LM, Turetsky DM, Choi DW. Measurement of intracellular free zinc concentrations accompanying zinc-induced neuronal death. J Neurosci. 1999; 19:RC31. [PubMed: 10493776]
181. Snitsarev V, Budde T, Stricker TP, Cox JM, Krupa DJ, Geng L, Kay AR. Fluorescent detection of Zn^{2+}-rich vesicles with zinquin: Mechanism of action in lipid environments. Biophys J. 2001; 80:1538–1546. [PubMed: 11222314]
182. Cheng C, Reynolds IJ. Calcium-sensitive fluorescent dyes can report increases in intracellular free zinc concentration in cultured forebrain neurons. J Neurochem. 1998; 71:2401–2410. [PubMed: 9832138]
183. Zalewski PD, Forbes IJ, Betts WH. 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. 1993; 296:403–408. [PubMed: 8257431]
184. Que EL, Domaille DW, Chang CJ. Metals in neurobiology: probing their chemistry and biology with molecular imaging. Chem Rev. 2008; 108:1517–1549. [PubMed: 18426241]
185. Simons TJ. Measurement of free Zn^{2+} ion concentration with the fluorescent probe mag-fura-2 (furaptra). J Biochem Biophys Methods. :25–37. 1993/08/01 ed1993.
186. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem. 1985; 260:3440–3450. [PubMed: 3838314]
187. Stork CJ, Li YV. Intracellular zinc elevation measured with a “calcium-specific” indicator during ischemia and reperfusion in rat hippocampus: a question on calcium overload. J Neurosci. 2006; 26:10430–10437. [PubMed: 17035527]
188. Tsien R, Pozzan T. Measurement of cytosolic free Ca^{2+} with quin2. Methods Enzymol. 1989; 172:230–262. [PubMed: 2747529]
189. Gee KR, Zhou ZL, Qian WJ, Kennedy R. Detection and imaging of zinc secretion from pancreatic beta-cells using a new fluorescent zinc indicator. J Am Chem Soc. 2002; 124:776–778. [PubMed: 11817952]
190. Naik HB, Beshire M, Walsh BM, Liu J, Soybel DI. Secretory state regulates Zn^{2+} transport in gastric parietal cell of the rabbit. Am J Physiol Cell Physiol. 2009; 297:C979–C989. [PubMed: 19675302]
191. Devinney MJ 2nd, Reynolds IJ, Dineley KE. Simultaneous detection of intracellular free calcium and zinc using fura-2FF and FluoZin-3. Cell Calcium. 2005; 37:225–232. [PubMed: 15670869]
192. Kiedrowski L. Cytosolic acidification and intracellular zinc release in hippocampal neurons. J Neurochem. 2012; 121:438–450. [PubMed: 22339672]

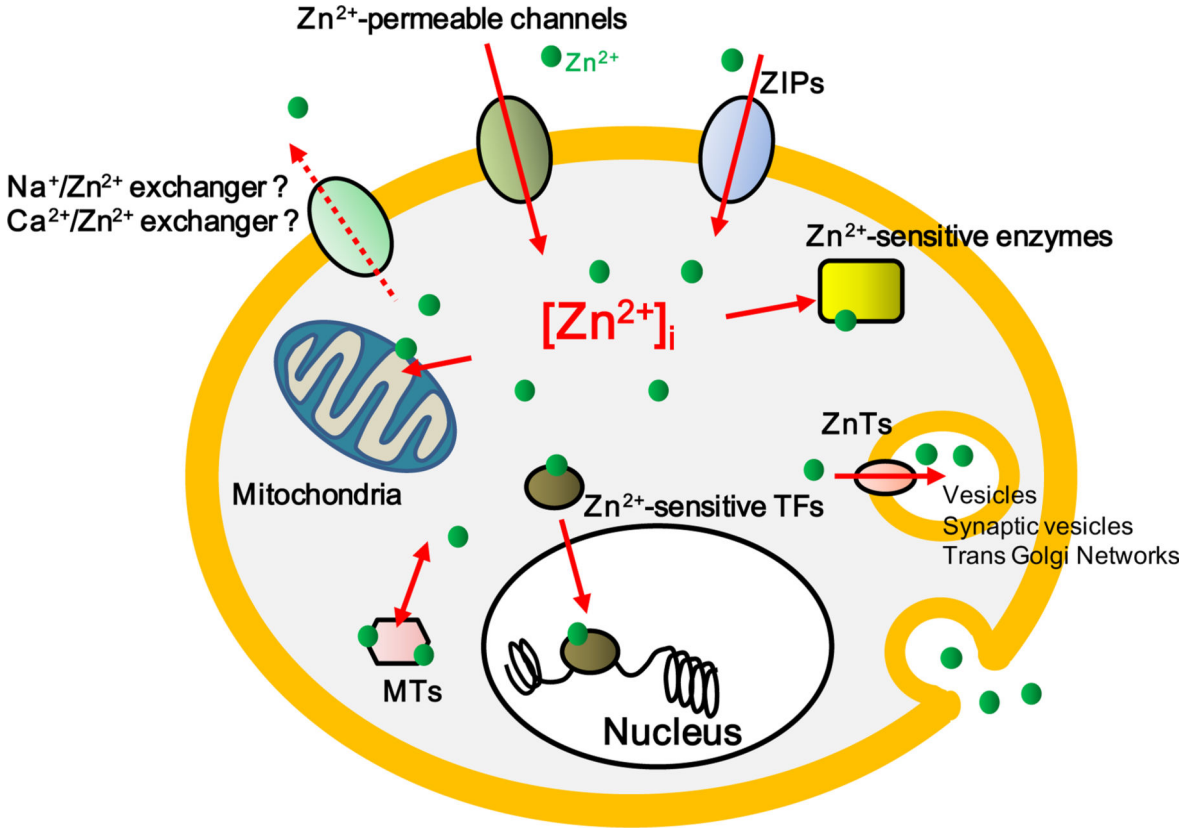

Figure 1.

Schematic illustration of the molecular mechanisms involved in intracellular Zn^{2+} homeostasis. Over 90% of intracellular Zn^{2+} binds to molecules. The remaining loosely bound Zn^{2+} affects the equilibrium state and zinc signaling. A fraction of the variable free Zn^{2+} is brought through Zn^{2+}-permeable ion channels. Excessive accumulation of labile Zn^{2+} disrupts cellular Zn^{2+} homeostasis, resulting in cellular dysfunction. Hypothetical exchangers are depicted with a dashed arrow.

Table 1

Zinc-permeable channels in vertebrates

Channels/receptors	Cells/organs/tissues	Endogenous function & significance	References
VDCCs	muscle, neurons, mast cells, pancreatic b cells	Zn^{2+} toxicity in neurons is enhanced by high K^{+}. Zinc wave mediated by L-type calcium channel affects expression of IL-6 and TNFα genes.	[78, 80-82, 88]
NMDA-Rs	neurons	Zn^{2+} toxicity is enhanced by NMDA. Field excitatory postsynaptic potentials might be enhanced by Zn^{2+} entry through NMDA-Rs.	[94, 97]
AMPA-Rs	neurons	Zn^{2+} toxicity is enhanced byAMPA.	[34, 82, 84]
nAch-Rs	muscle endplate, chromaffin cells	N/A	[108, 110]
TrpM6/7	Ubiquitous (TrpM7) Kidney (TrpM6)	Zn^{2+} toxicity in neurons is inhibited by silencing TrpM7 in neurons.	[116, 118, 120, 121, 123, 126]
TrpM3	Pancreatic b cells	Zn^{2+} is possibly taken up for subsequent exocytosis.	[134]
TrpA1	DRG neurons	Zn^{2+} causes nociception through TRPA1	[142]
TrpV6	Intestine, kidney, placenta	N/A	[110]
TrpC6	Ubiquitous	N/A	[157]
TRPML1	Ubiquitous	TRPML1 knockout results in abnormal lysosomal Zn^{2+} homeostasis.	[166, 167]