Chapter 5
Zinc Signaling by "Zinc Wave"

Keigo Nishida and Satoru Yamasaki

Abstract Zinc (Zn) is an essential heavy metal for all organisms. Zn homeostasis is maintained in mammalian cells through the activity of Zn transporters and Zn-permeable channels and through metallothionein expression levels. Zn is important in nucleic acid metabolism, cell replication, and tissue growth and repair. Zn deficiency is associated with a wide range of pathological conditions, such as impaired immunity, growth retardation, disorders in brain development, and delayed wound healing. Zn binds and affects the activity of several signaling molecules and of transcription factors that have a Zn-binding motif. However, whether Zn itself, as does calcium, acts as an intracellular signaling molecule has been a point of speculation. Recently, we and other groups have demonstrated that Zn does indeed act as an intracellular signaling molecule, converting extracellular stimuli to intracellular signals and controlling various cell functions. This chapter summarizes our current understanding of Zn signaling, especially with regard to the *Zn wave* and the role of Zn signaling in immune cells, and discusses how these processes contribute to allergic responses.

Keywords Immune response • Mast cell • Second messenger • Signal transduction • Zinc • Zinc transporter • Zinc wave

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5.1 Introduction

Zinc (Zn), an essential trace element, is an important structural component in a great number of proteins, including intracellular signaling enzymes and transcription factors (Prasad 1995; Vallee and Auld 1993). The wide-ranging effects of Zn in the immune and nervous systems have been demonstrated both in vivo and in vitro, and these effects mainly depend on Zn concentration (Frederickson et al. 2005; Rink and Gabriel 2000). Many researchers have reported that Zn depletion decreases immune function. Natural killer cell-mediated cytotoxic activity, antibody-mediated responses, and host defenses against pathogens and tumors are all reduced in Zn-deficient mice (Fernandes et al. 1979; Fraker et al. 1982; Keen and Gershwin 1990).

Zn plays an essential constitutive role in the conformation and activity of many enzymes, transcription factors, signaling molecules, and other components involved in cellular processes. However, high concentrations of Zn can be cytotoxic, and can induce apoptosis in T and B cells (Ibs and Rink 2003; Telford and Fraker 1995). Zn concentration and distribution within and between cells are controlled by Zn transporters, such as those in the Slc39/ZIP and Slc30/ZnT families, which increase and decrease intracellular Zn levels, respectively (Fukada and Kambe 2011; Kambe et al. 2004; Lichten and Cousins 2009), and Zn is buffered by Zn-binding molecules (Palmiter 2004; Vallee 1995).

We recently showed that Fc epsilon receptor I (FcεRI) stimulation induces an increase in intracellular free Zn, and we named this phenomenon the "Zn wave" (Yamasaki et al. 2007). The Zn wave occurs within several minutes after FcεRI stimulation and originates from the endoplasmic reticulum (ER). Thus, extracellular stimuli can affect intracellular Zn levels through Zn transporters and Zn-permeable channels, and Zn acts as an intracellular signaling molecule (Taylor et al. 2012; Yamasaki et al. 2012). We have proposed that intracellular Zn signaling can be classified into at least two categories: late Zn signaling, which depends on transcriptional changes in Zn-transporter expression, and early Zn signaling, which involves the Zn wave, in which Zn is directly released from the cell organelle by an extracellular stimulus such as FcεRI (Fukada et al. 2011; Hirano et al. 2008; Murakami and Hirano 2008; Nishida et al. 2011).

Many studies have shown that Zn is important in the immune system and that imbalances in Zn homeostasis lead to various disorders, but several questions remain as to how Zn homeostasis and signaling are regulated in mast and other immune cells, and whether Zn transporters are involved in immune cell function. In this chapter, we briefly describe aspects of the Zn-wave phenomenon and discuss the role of Zn signaling in allergic responses.

5.2 Cellular Zn Homeostasis by Zn Transporters and Metallothioneins

Computational analysis suggests that approximately 10 % of the genes in the human genome may contain Zn-binding motifs (Andreini et al. 2006), reflecting the physiological relevance of Zn and Zn-binding proteins for life in general. In fact, Zn has wide-ranging effects on cellular functions (Vallee and Falchuk 1993), and imbalances in Zn homeostasis can impair growth and cause hair loss, thickening and hyperkeratinization of the epidermis, and testicular atrophy in humans and animal models (Prasad 1991). The concentration and distribution of both intracellular and extracellular Zn are controlled via buffering by Zn-binding molecules, such as metallothioneins, and by Zn transporters, including Slc39/ZIP and Slc30/ZnT family members, which increase or decrease the intracellular Zn levels, respectively (Fig. 5.1).

Metallothioneins (MTs), small cysteine-rich proteins that bind Zn and other metal ions, are thought to be responsible for regulating intracellular Zn concentrations and detoxifying nonessential heavy metals. When intracellular free Zn reaches a threshold concentration, activation of the Zn sensor MTF-1 induces the expression of MTs, which sequester Zn ions (Andrews 2001). Thus, MTs serve as a biochemical device that controls free Zn concentrations by sequestering or releasing Zn in response to oxidative signaling and other biochemical events.

5.3 Zn Signaling

Cytosolic and cellular concentrations of free Zn change dynamically in many cells in response to various stimuli. Three major sources for cytosolic free Zn have been identified: uptake from the extracellular environment, reversible storage in vesicles,

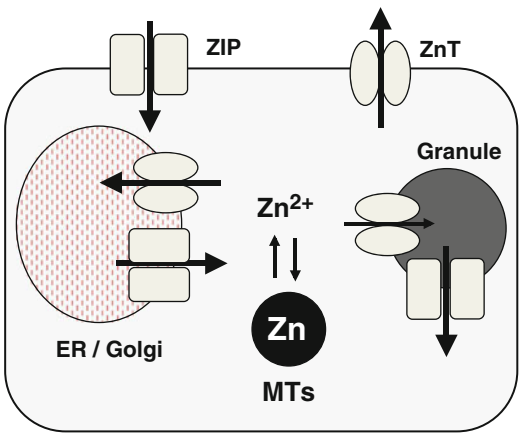

Fig. 5.1 Subcellular localization of Zn transporters and metallothioneins. An illustration of the subcellular localization and potential functions of ZIP- and ZnT family members, based on currently available information. *Arrows* indicate the predicted direction of Zn mobilization. *MTs* metallothionein1/2, *ER* endoplasmic reticulum

and oxidative release from storage proteins. Thus, Zn is increasingly recognized as a potential signaling molecule (Frederickson and Bush 2001; Haase and Rink 2009; Hirano et al. 2008; Sensi et al. 2009).

5.3.1 Zn Acts as a Neurotransmitter

Zn is highly concentrated in synaptic vesicles and is released with glutamate in an activity-dependent manner (Assaf and Chung 1984). Recently, Zn imaging techniques that allow the real-time analysis of Zn concentration and distribution using fluorescent sensor molecules have been widely applied (Kikuchi 2010). Microfluorescence imaging of Zn secretions in rodents showed that exocytotic presynaptic stimulation prompts the release of Zn from hippocampal mossy fiber terminal vesicles into the surrounding milieu (Li et al. 2001; Qian and Noebels 2005; Ueno et al. 2002), where it is taken up into the cytoplasm of neighboring cells through gated Zn channels. A rapid influx of Zn through calcium ion-permeable AMPA/kainate (Ca-A/K) channels triggers the generation of reactive oxygen species (ROS) and is potentially neurotoxic (Weiss and Sensi 2000).

In the foregoing scenario, Zn activity is similar to that of neurotransmitters, which are stored in membrane-enclosed synaptic vesicles, are released by exocytosis, and activate postsynaptic cells through transmitter-gated ion channels (Colvin et al. 2003; Hershfinkel et al. 2001; Xie and Smart 1994). Because Zn modulates both the current response (mediated by excitatory and inhibitory neurotransmitter receptors) and the efficacy of transporter-driven neurotransmitter re-uptake (Smart et al. 2004), synaptically released Zn has been proposed to function as an important regulator of synaptic transmission and plasticity (Lu et al. 2000; Vogt et al. 2000).

ZnT3/Slc30a3, which is highly expressed on synaptic vesicle membranes, is essential for Zn uptake into the vesicles. Although *ZnT3*-KO mice show a loss of stainable Zn in synapses (Cole et al. 1999), they are behaviorally normal except for an enhanced susceptibility to kainite-induced seizures (Lopantsev et al. 2003). In the Tg2576 transgenic mouse model for Alzheimer's disease (AD), Zn release during synaptic transmission induces cerebral β-amyloid (Aβ) deposits. When these mice are crossed with the *ZnT3*-KO mice, which lack synaptic Zn, the cerebral Aβ deposition is nearly abolished in the Tg2576/*ZnT3*$^{-/-}$ progeny (Lee et al. 2002). A recent study by Tamaki et al. provides evidence that Zn is co-secreted with insulin in a ZnT8-dependent manner, and that the secreted Zn not only affects neighboring endocrine cells, but also plays an important role in hepatic insulin clearance by inhibiting clathrin-dependent insulin endocytosis (Tamaki et al. 2013). This finding suggests that Zn release is involved in various pathologies, such as AD and diabetes.

Recent studies have shown that Zn is an endogenous agonist for GPR39, an orphan receptor that is structurally and functionally related to G protein-coupled receptors. The concept of GPR39 as a Zn-sensing receptor in the brain is consistent with the role of Zn as a neurotransmitter (Besser et al. 2009; Yasuda et al. 2007).

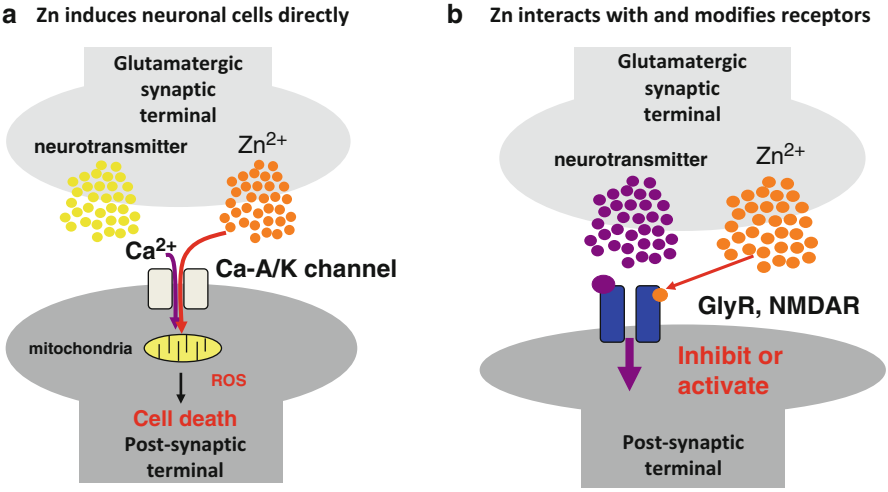

Fig. 5.2 Zn acts as a neurotransmitter. Zn behaves as a neurotransmitter in neuronal cell communication. **a** Zn is released from vesicles into the surrounding milieu upon exocytotic presynaptic stimulation, and is then taken up into the cytoplasm of neighboring cells through gated Zn channels. The rapid influx of Zn through calcium (Ca) ion-permeable AMPA/kainate (Ca-A/K) channels triggers the generation of reactive oxygen species (ROS) and is potentially neurotoxic. **b** Zn released from glutamatergic synaptic vesicles can bind to the Zn-binding sites of GlyR and NMDAR on postsynaptic neurons to modulate neurotransmission

Intercellular Zn communication mediated through the Zn-sensing receptor GPR39 is also involved in regulating epithelial cell repair (Sharir et al. 2010) and endocrine pancreatic function (Holst et al. 2009). Also supporting the role of Zn as a neurotransmitter, Hosie et al. identified two Zn-binding sites and characterized a third in the GABA receptor using site-directed mutagenesis (Hosie et al. 2003). Hirzel et al. produced knock-in mice carrying a constructed Zn-binding site (a D80A mutation) in the glycine receptor alpha-1-subunit gene (*Glra1*) and demonstrated that Zn modulates neurotransmission (Hirzel et al. 2006). Zn is also known to inhibit *N*-methyl-D-aspartate receptor (NMDAR) activity through a dual mechanism: a voltage-dependent channel block, and a voltage-independent reduction in the probability of the channel opening (Christine and Choi 1990; Legendre and Westbrook 1990; Mayer and Vyklicky 1989). Thus, Zn appears to act as a neurotransmitter in addition to its other roles in the neural system (Fig. 5.2).

5.3.2 Zn Acts as a Second Messenger

cAMP was the first intracellular second messenger to be discovered (Berthet et al. 1957), and calcium was the second. At present, only a limited number of intracellular signaling effectors are known, including cAMP, calcium, NO, lipid

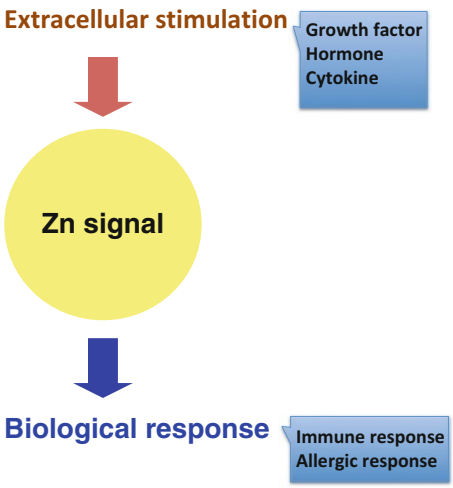

Fig. 5.3 Zn as an intracellular second messenger candidate. Second messengers are molecules that receive signals from stimuli such as hormones, growth factors, and cytokines at the cell surface and relay these signals to target molecules in the cytosol or nucleus. This action, in turn, induces intracellular events that can regulate various biological responses. Zn functions are consistent with those of second messenger molecules

mediators, and G proteins (Gomperts et al. 2002). Interestingly, Zn has also been recognized to have an essential role as a second messenger. Zn itself affects a variety of signaling molecules, including PKC, Ca/calmodulin-dependent protein kinase II (CaMKII), Erk1/2, cAMP-dependent protein kinase (PKA), protein tyrosine phosphatase, and caspase-3 (Brautigan et al. 1981; Haase and Maret 2005; Hubbard et al. 1991; Lengyel et al. 2000; Murakami et al. 1987; Park and Koh 1999; Perry et al. 1997). In addition, Zn activates ion channels such as the transient receptor potential ankyrin 1 (TRPA1) (Andersson et al. 2009; Hu et al. 2009), ATP-sensitive K^+ (Prost et al. 2004), and large-conductance Ca-activated K^+ (Hou et al. 2010) channels. Together, these findings suggest that Zn may function as an intracellular signaling molecule or second messenger if extracellular stimuli, such as cytokines or growth factors, cause the intracellular Zn status to change, whether dependently or independently of transcriptional changes in MTs or Zn transporters (Fig. 5.3).

It is important to determine whether Zn transporters are involved in Zn signaling. We reported that the ZIP6/SLC39A6 is required for the epithelial-mesenchymal transition (EMT) in the zebrafish gastrula organizer, as it is essential for nuclear retention of the E-cadherin-repressor Snail (Yamashita et al. 2004). Because ZIP6/SLC39A6 expression in the zebrafish organizer is dependent on STAT3 activation, any extracellular stimulus that regulates STAT3 activation could change the intracellular Zn level by inducing changes in ZIP6/SLC39A6 or other Zn transporters. TLR4 decreases the intracellular free Zn level by inducing changes in Zn transporter expression in dendritic (Kitamura et al. 2006) and pulmonary endothelial cells (Thambiayya et al. 2011). These observations support the role of Zn as an intracellular second messenger, that is, as a molecule whose intracellular status is altered in response to an extracellular stimulus and that is capable of transducing the extracellular stimulus into an intracellular signaling event.

The role of Zn as a second messenger is further supported by our findings that ZIP13/Slc39a13 is required for the BMP/TGF-β-induced nuclear localization of Smad proteins (Fukada et al. 2008), that ZIP14/Slc39a14 is involved in GPCR-mediated signal transduction through cAMP basal-level regulation (Hojyo et al. 2011), and that FcεRI-stimulation-induced PKC activation is dependent on ZnT5/Slc30a5 in mast cells (Nishida et al. 2009). Thus, extracellular stimuli can, by changing the expression of Zn transporters, affect intracellular signaling pathways by changing the intracellular Zn status.

Besides Zn signaling mediated by Zn transporter expression, Zn signaling is also exemplified by the Zn wave phenomenon, which is a transcription-independent increase in intracellular Zn that occurs in mast cells relatively rapidly (several minutes) after extracellular stimulation (Yamasaki et al. 2007). Zn waves, which originate from the ER, depend on calcium influx and Erk1/2 activation in mast cells. Because the Zn wave does not involve extracellular Zn, and is induced within several minutes of FcεRI stimulation, Zn here acts as an intracellular signaling molecule. Using the Zn probe FluoZin-3, Haase et al. revealed that the intracellular Zn level is elevated in peripheral blood mononuclear cells (PBMCs) after polymethyl acrylate (PMA) stimulation (Haase et al. 2006), and in human leukocytes, especially monocytes, after physiological stimulation (Haase et al. 2008). In addition, ZIP7/SlC39A7 is reported to regulate intracellular Zn at the ER membrane (Taylor et al. 2008). It is also possible that transporters and channels other than ZIP or ZnT family members generate Zn waves in individual cell types with various types of stimulation. In any case, the precise molecular mechanisms generating the Zn wave have not been clarified.

5.3.3 Zn-Signaling Gatekeepers

Recently, three independent groups reported rapid increases in free Zn, as seen in the Zn wave, in mast cells and lymphocytes upon stimulation with antigens such as FcεRI, B-cell receptors (BCRs), and T-cell receptors (TCRs) (Taniguchi et al. 2013; Yamasaki et al. 2012; Yu et al. 2011). Interestingly, these studies reported that the dynamic changes in cytosolic free Zn were regulated by Zn-permeable channels and Zn transporters.

In this section, we briefly describe gatekeepers of Zn signaling, their regulation, and their molecular targets.

5.3.3.1 LTCC-Mediated Zn Signaling in Mast Cells

L-type calcium channels (LTCCs) conduct Zn and can act as Zn-permeable channels on the plasma membrane of neurons and pancreatic β cells (Atar et al. 1995; Gyulkhandanyan et al. 2006; Sensi et al. 1997). However, whether LTCCs can function in releasing Zn from intracellular organs has not been clarified. It was

recently revealed that LTCCs have the potential to act as a gatekeeper for the Zn wave that occurs when FcεRI induces intracellular Zn signaling in mast cells (Yamasaki et al. 2012).

It is well established that LTCCs function as voltage-gated calcium channels on the plasma membrane. LTCC complexes include α_1-, β-, and α_2/δ-subunits. The α_1-subunit functions as the voltage sensor, selective filter, and ion-conducting pore (Catterall 2000). The α_1-subunit on the cell surface is thought to require an association with the β-subunit, which masks one or more ER retention signals (Bichet et al. 2000; Cornet et al. 2002).

In bone marrow-derived mast cells (BMMCs), the dominantly expressed α_1-subunit (α_{1D}) is *cacna1d*, the α_1-subunit for the LTCC. The expression of LTCC β-subunits, which are required for the localization of α_1-subunits to the plasma membrane, is very low in mast cells. Furthermore, mast cells express high levels of ZnT-1/Slc30a1, which interacts with β-subunits on the plasma membrane, reducing their availability to bind α_1 and inhibiting α_1-subunit trafficking to the plasma membrane (Levy et al. 2009). Therefore, α_{1D} in mast cells localizes to the intracellular area; it co-localizes partially with the ER marker calnexin but not with F-actin, which accumulates beneath the plasma membrane. These observations indicate that α_{1D} localizes preferentially to intracellular organelle membranes, such as the ER membrane, rather than to the plasma membrane. This intracellular localization of α_{1D} in BMMCs suggests that it plays a different role in BMMCs than in other cells, in which it is located on the plasma membrane and acts as a calcium channel.

Consistent with this idea, treating mast cells with verapamil, an LTCC inhibitor, reduces their Zn waves compared to control cells, without disturbing cell survival, FcεRI expression, or the FcεRI-mediated calcium elevation. Diltiazem, another type of LTCC antagonist, also inhibits Zn waves without disturbing the calcium elevation. Not only does verapamil have no effect on the FcεRI-mediated calcium elevation in BMMCs, but calcium-mediated signaling, such as the normal nuclear translocation of NFAT2 in response to calcium elevation, is also unaffected (Yamasaki et al. 2012).

On the other hand, treatment with the LTCC agonist (*s*)-(–)-BayK8644 without antigen stimulation elevates the intracellular Zn level but not the calcium level in mast cells (Yamasaki et al. 2012). These findings indicate that LTCC function in mast cells differs from its function as a calcium channel in other cell types, such as neurons and pancreatic β cells. LTCC may have little effect on the intracellular calcium regulation in mast cells because the main mode of calcium influx in these cells, as in lymphocytes, is store-operated calcium (SOC) entry (Vig and Kinet 2009). LTCC agonists induce an increase in intracellular Zn even in the absence of calcium, and this increase is inhibited by verapamil. FcεRI induces similar Zn increases in BMMCs in the presence or absence of an LTCC agonist, indicating that the mechanisms responsible for the FcεRI-induced Zn wave and the LTCC agonist-induced Zn elevation are probably similar.

Although the FcεRI-induced Zn wave is significantly reduced in α_{1D}-knockdown BMMCs, the FcεRI-induced calcium elevation is intact in these cells, as is the case

with verapamil-treated cells. Moreover, the ectopic expression of wild-type α_{1D} rescues the inhibitory effect of α_{1D} siRNA knockdown on the Zn wave. These results suggest that the LTCC α_{1D} subunit is a gatekeeper for the Zn wave.

This finding raises the question of how LTCC regulates the release of Zn from the ER into the cytoplasm. The α_1-subunit of LTCC contains a voltage-sensor domain, and channel activity is elevated after membrane depolarization. The plasma-membrane potential in BMMCs is hyperpolarized after FcεRI stimulation (Shumilina et al. 2008; Vennekens et al. 2007). However, inhibiting the FcεRI-mediated plasma-membrane hyperpolarization by high KCl treatment does not impair the induction of the Zn wave. Treatment with bongkrekic acid, which inhibits ADP/ATP transport, inhibits FcεRI-mediated intracellular membrane depolarization but does not inhibit the induction of the Zn wave. These observations suggest that regulation of the membrane potential might not affect the Zn wave generation.

Phosphorylation of the pore-forming α_1-subunit has an additional effect on channel activity; in fact, cAMP-mediated channel activity is reduced by site-directed mutagenesis of the PKA consensus sites of α_{1D} (Ramadan et al. 2009). However, a PKA inhibitor did not inhibit the Zn wave. Therefore, PKA might not assist in regulating the Zn wave, at least not in mast cells. This event may be controlled by as yet unidentified regulatory proteins and mechanisms on the ER membrane.

These results show that the LTCC pore-forming α_{1D}-subunit, when expressed on the ER membrane, functions as a gatekeeper for the Zn wave in mast cells, and that this LTCC-mediated Zn wave may function as an intracellular Zn signal that can positively modify signal transduction to produce inflammatory cytokines (Fig. 5.4).

5.3.3.2 ZIP9-Mediated Zn Signaling in B Cells

Taniguchi et al., using the DT40 chicken B-cell line as a model, found that the Zn transporter ZIP9/SLC39A9 induces an increase in intracellular Zn. In contrast to the Zn wave, which originates from the ER, the source of intracellular free Zn in these chicken ZIP9-knockout DT40 (cZip9KO) cells is thought to be the Golgi bodies. Consistent with this scenario, ZIP9 is thought to function in releasing Zn from the Golgi bodies to the cytosol (Taniguchi et al. 2013).

The BCR-signaling pathway is critical for many cellular events, including cell growth, cell proliferation, and apoptosis (Dal Porto et al. 2004; Harwood and Batista 2008, 2010). BCR activation transduces the signal to several cascades, including the PI-3K/Akt, PLCγ2/PKC, and Ras/Raf/ERK cascades (Brazil and Hemmings 2001; Hashimoto et al. 1998; Kurosaki 2011). These cascades are important for the differentiation of antibody-producing cells and memory B cells. ZIP9-mediated Zn signaling affects BCR-mediated Akt and ERK phosphorylations. In fact, BCR-induced Akt and ERK phosphorylations are significantly decreased, along with the enzymatic activity of protein tyrosine phosphatase (PTPase), in cZip9KO cells. Consistent with this observation, overexpressing hZIP9 decreases

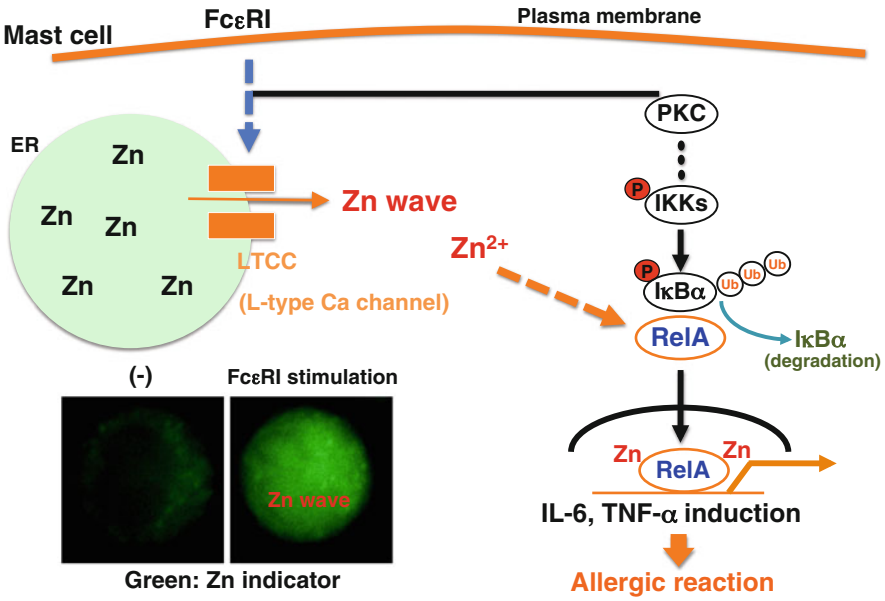

Fig. 5.4 The Zn wave in FcεRI-mediated mast cell activation. In a phenomenon we named the *Zinc wave*, FcεRI stimulation induces the rapid elevation of intracellular Zn in mast cells by releasing Zn from the ER at the perinuclear region. The LTCCα_{1D} subunit expressed on the ER membrane acts as the Zn-wave gatekeeper in mast cells. LTCC-mediated Zn waves positively regulate the NF-κB DNA-binding activity and are involved in regulating cytokine production

the PTPase activity in Zn-treated cZip9KO cells (Taniguchi et al. 2013). At present, it is not known how ZIP9-mediated Zn signaling is regulated by BCR stimulation. ZIP7 is reported to be activated by its phosphorylation by protein kinase CK2 in a human breast cancer cell line (Taylor et al. 2012). This finding raises the possibility that CK2 can phosphorylate ZIP9 and regulate ZIP9-mediated Zn signaling.

This study concluded that ZIP9 on the Golgi body membrane regulates cytosolic Zn, enhancing the Akt and ERK phosphorylations in B cells (Fig. 5.5). Thus, Zn signaling in B cells may occur via a mechanism similar to that of the Zn wave in mast cells.

5.3.3.3 ZIP6-Mediated Zn Signaling in T Cells

Yu et al. found Zn signaling in T cells; TCR stimulation triggers an increase in cytoplasmic Zn concentration within 1 min (Yu et al. 2011). This increase is dependent on extracellular Zn concentration, suggesting that TCR stimulation induces an influx of extracellular Zn into the T cell. Moreover, this influx of Zn is inhibited by silencing the Zn transporter ZIP6, which is expressed on the cytoplasmic membrane.

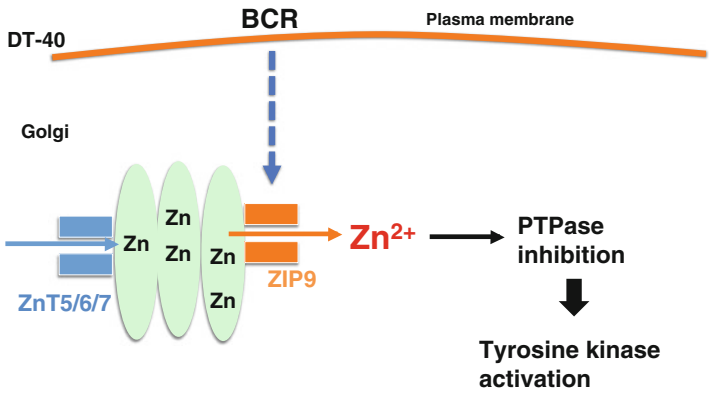

Fig. 5.5 Proposed action sites of intracellular Zn release by ZIP9 in the activation of B-cell receptor signaling in DT40 cells. An illustration of the proposed mechanism by which Zn induces the inhibition of protein tyrosine phosphatase (PTPase) by ZIP9 in DT40 cells, leading to the activation of B-cell receptor (*BCR*) signaling. Intracellular Zn is incorporated into Golgi bodies by ZnT5/6/7, and ZIP9 induces its release from the Golgi back into the cytosol. This Zn inhibits PTPase activity and induces tyrosine kinase activation probably indirectly by regulating upstream components of the signal transduction

Early TCR signaling events include the tyrosine phosphorylation of several signaling molecules. The Src protein kinase Lck is primarily responsible for the early phosphorylation of tyrosines within the ITAM motifs of CD3ζ and ZAP70 (Palacios and Weiss 2004). Extracellular Zn influences ZAP70 phosphorylation and inhibits negative regulatory feedback loops, accounting at least in part for the increase in ZAP70 phosphorylation. SHP-1, which dephosphorylates tyrosines within the ITAM motifs of ZAP70 and other signaling molecules after being recruited to Lck (Altan-Bonnet and Germain 2005), is a prime candidate target for the ZIP6-mediated Zn signaling. In fact, an increase in Zn influx reduces SHP-1 recruitment to the TCR activation complex, augments ZAP70 phosphorylation, and sustains calcium influx. Thus, Yu et al. proposed that the influx of Zn after TCR stimulation leads to a local increase in cytoplasmic Zn that modifies early TCR signaling events (Fig. 5.6).

5.4 Zn and Zn Signaling in Allergies

Many studies have reported that Zn depletion decreases immune function, indicating that Zn acts as a positive regulator in immune responses. However, the precise roles of Zn and the molecular mechanism(s) of its function in allergic responses have not been clarified. Here, we describe the effect of Zn and Zn homeostasis on biological events, especially those of mast cell-mediated allergy responses, and outline our current understanding of the physiological role of the Zn wave.

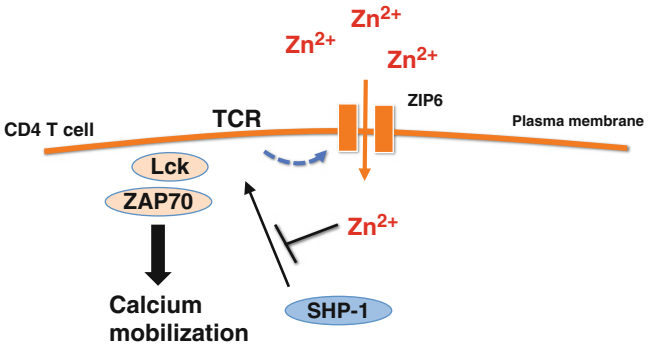

Fig. 5.6 Zn functions as an ionic signaling molecule after T-cell activation. Cytoplasmic Zn concentrations increase within 1 min after T-cell receptor (*TCR*) triggering as a result of Zn influx via the transporter ZIP6. This increase, which is most pronounced in the immediate subsynaptic area, enhances TCR signaling, at least partly by inhibiting the recruitment of SHP-1

5.4.1 The role of Zn in Mast Cell-Mediated Allergic Responses

Allergy-related cells, such as mast cells, eosinophils, and basophils, are involved in allergic reactions such as anaphylaxis, asthma, and atopic dermatitis (Galli et al. 2008; Kawakami et al. 2009; Metz et al. 2007). Mast cell activation leads to the secretion of two classes of mediators. The first consists of preformed mediators that are stored in granules and can be quickly secreted in activated cells. The second class of mediators, the cytokines and chemokines, must be newly synthesized and are secreted more slowly. These released molecules play pivotal roles in the inflammatory reactions observed in patients with allergies.

Intracellular Zn levels and distribution can be analyzed using the Zn probe Zinquin, which allows the imaging of distinct pools of Zn in allergy-related cells. Granules in mast cells, for instance, fluoresce intensely with Zinquin (Ho et al. 2004). Airway epithelial cells are also rich in Zn (Truong-Tran et al. 2000). Furthermore, Zn deficiency is reported to increase allergic eosinophilic inflammation, whereas dietary Zn supplementation attenuates its intensity (Richter et al. 2003a). Interestingly, Zn deficiency is a risk factor for asthma development (Riccioni and D'Orazio 2005; Zalewski et al. 2005). These reports suggest that Zn is involved in the development of allergic disease. In addition, high levels of *ZIP2* are observed in the leukocytes of asthmatic infants (Xu et al. 2009). However, the precise role of Zn and Zn transporters in allergy-related cells is poorly understood.

We showed that Zn is required for both degranulation and cytokine production in mast cells. The Zn chelator TPEN [*N*,*N*,*N*,*N*-tetrakis (2-pyridylmethyl) ethylenediamine] inhibits histamine release, cytokine production, and lipid mediator secretion. The inhibitory effects of TPEN are rescued by Zn supplement, and chelators of other metals do not affect mast cell function (Kabu et al. 2006). These observations indicate that Zn is crucial for degranulation and cytokine production in mast cells. Similarly, it has been reported that Zn depletion by TPEN or by the

clinically used heavy metal chelator DMPS (Torres-Alanis et al. 1995) inhibits the mRNA expression of eotaxin and other chemokines in human lung cell lines (Richter et al. 2003b). All together, these reports suggest that Zn chelators and their derivatives are a promising source of anti-allergy drugs that may act differently from histamine antagonists and other currently available allergy treatments.

It is not clear exactly how Zn chelators inhibit mast cell function. We reported that the degranulation of mast cells can be divided into two processes. First, FcεRI stimulation triggers microtubule polymerization and granule translocation to the plasma membrane in a calcium-independent manner. Fyn/Gab2/RhoA signaling, but not Lyn/SLP-76 signaling, is critical in this calcium-independent microtubule-dependent pathway (Nishida et al. 2005). Second, the granules fuse with the plasma membrane in a well-characterized calcium-dependent manner. To clarify the mechanisms behind the effect of Zn chelators, we first examined whether TPEN interfered with either of these two processes. Although TPEN had little effect on calcium mobilization or the early FcεRI-induced tyrosine phosphorylation of various signaling molecules, it suppressed the FcεRI-induced granule translocation. Given that granule translocation depends on cytoskeletal proteins such as tubulin and actin (Goode et al. 2000), and that microtubules are critical for granule translocation and vesicle transport (Nishida et al. 2005; Smith et al. 2003), we speculated that TPEN might affect microtubule assembly. However, such an effect by TPEN on FcεRI-induced microtubule formation has not been found, suggesting the existence of Zn-regulated molecule(s) that are directly linked to microtubules and granules. Kinesin receptors, or linker-cargo proteins, have been identified as key molecules for microtubule-dependent vesicle trafficking (Schnapp 2003); thus, the TPEN target might have a kinesin-interacting region through which it links kinesin to vesicles. In addition, TPEN suppresses FcεRI-mediated cytokine production and the transcription of interleukin (IL)-6 and tumor necrosis factor (TNF)-α mRNAs. PKC is activated upon FcεRI stimulation and is involved in cytokine production through NF-κB activation (Klemm et al. 2006; Nechushtan et al. 2000), and TPEN inhibits the FcεRI-mediated plasma membrane translocation of PKC (Kabu et al. 2006). These results suggest PKC as one of the TPEN targets in regulating cytokine production. This hypothesis is supported by findings from other groups showing that the Zn-binding domain of PKC is required for PKC translocation to the plasma membrane after stimulation (Oancea et al. 1998).

We demonstrated that ZnT5 is crucial in mast cell activation and mast cell-mediated allergic reactions. Znt5 is expressed at high levels in mast cells, and its transcription is enhanced by FcεRI stimulation, suggesting that Znt5 is involved in mast cell-mediated allergic reactions. *ZnT5-KO* mice have defects in mast cell-mediated, delayed-type allergic reactions such as contact hypersensitivity, but not in immediate-type reactions such as anaphylaxis (Nishida et al. 2009). Consistent with this in vivo analysis, ZnT5 is required for FcεRI-mediated cytokine production, but not for degranulation, in mast cells. In *ZnT5-KO* mast cells, the FcεRI-induced IL-6 and TNF-α mRNAs are reduced. Finally, we showed that ZnT5 is required for the FcεRI-induced translocation of PKC to the plasma membrane and for the nuclear translocation of NF-κB. Thus, ZnT5 is selectively required for mast cell-mediated, delayed-type allergic responses, and is a novel player in PKC/NF-κB

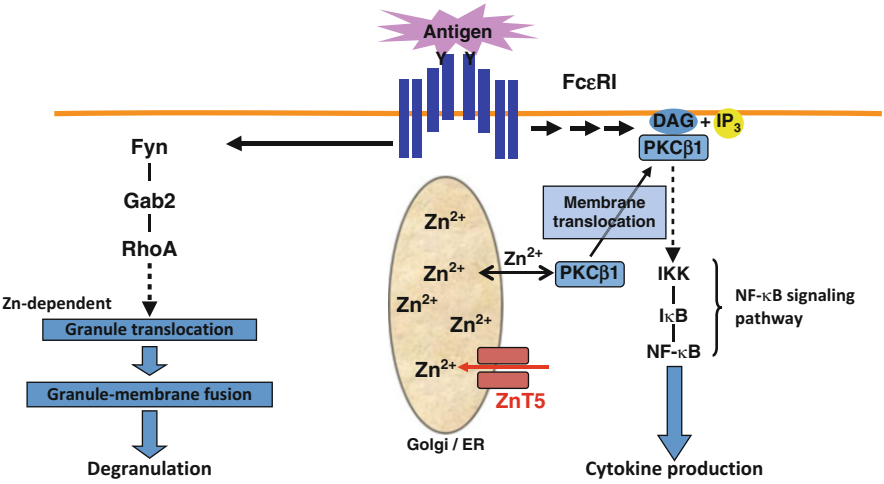

Fig. 5.7 Zn and Zn transporters are involved in FcεRI-mediated mast cell activation. Zn is required in multiple steps of FcεRI-induced mastcell activation, including degranulation and cytokine production. Zn levels depend on FcεRI-induced granule translocation, regulated by the Fyn/Gab2/RhoA-mediated signaling pathway. Zn and ZnT5 are also required for PKC translocation to the plasma membrane and NF-κBs subsequent nuclear translocation, leading to the production of cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α

signaling. How ZnT5 regulates PKC translocation to the plasma membrane is still unknown. PKC contains a Zn-binding motif, and Zn is essential for maintaining the structure of PKC (Corbalan-Garcia and Gomez-Fernandez 2006). Mutational analysis showed that the Zn-binding domain of PKC is essential for the plasma membrane translocation of PKC. Furthermore, in experiments utilizing *ZnT5-KO* DT40 cells, Suzuki and colleagues showed that ZnT5 expressed on the ER–Golgi membrane is required for the enzymatic activity of the Zn-dependent alkaline phosphatases (ALPs), which are processed from apoALPs to holoALPs in the ER–Golgi (Suzuki et al. 2005a, b). Together, these findings indicate that ZnT5 may be involved in supplying Zn to the Zn finger-like domain in PKC and ALP.

All these findings suggest that Zn and Zn transporters are involved in mast cell-mediated allergic responses by regulating degranulation and cytokine production, and that Zn transporters modulate the PKC/NF-κB signaling pathway, which regulates the gene expression levels of cytokine and chemokines (Fig. 5.7).

5.4.2 *Role of the Zn Wave in Mast Cell-Mediated Allergic Responses*

As already mentioned, we found that the pore-forming α_{1D}-subunit of LTCC on the ER membrane is involved in generating the Zn wave in mast cells. Using siRNA or an LTCC antagonist, we found that the Zn wave can regulate cytokine gene

induction. LTCC antagonist-treated mast cells cannot increase the intracellular concentration of free Zn, either through a Zn wave or through the mRNA induction and protein synthesis of IL-6 and TNF-α. Furthermore, both FcεRI-induced Zn waves and cytokine gene induction are inhibited in mast cells treated with siRNA against the LTCC α_{1D}-subunit. These results suggest that the Zn wave is required for the FcεRI-induced cytokine production in mast cells.

It is not certain how the Zn wave acts to induce cytokine genes. We found that the LTCC-mediated intracellular Zn signal upregulates NF-κB DNA-binding activity and transactivation of inflammatory cytokines. This NF-κB-mediated transactivation can be divided into three steps: NF-κB first dissociates from IκB after IκB phosphorylation and degradation, then translocates from the cytosol to the nucleus, and finally binds to its target sequences. We found that the frequency of NF-κB p65 nuclear translocation is reduced in LTCC antagonist-treated cells, even though upstream regulators are unaffected. Treatment with the exportin inhibitor LMB enhances the amount of NF-κB in nuclei in both LTCC antagonist-treated and LMB-treated cells, suggesting that the Zn wave is not involved in NF-κB nuclear translocation. However, our evidence indicates that the Zn wave is required for NF-κB DNA-binding activity. In further support of this scenario, LTCC antagonist treatment reduces NF-κB DNA-binding activity, whereas supplementing cell lysates with Zn enhances it. These findings suggest that Zn wave-induced increases in intracellular Zn positively regulate NF-κB DNA-binding activity (Fig. 5.4).

Finally, we evaluated the role of the Zn wave in allergic reactions in vivo using an LTCC antagonist. Mast cells are effector cells for allergic responses in vivo, and mast cell-derived cytokines, which are induced by the FcεRI-mediated activation of the PKC/Bcl10/Malt1/NF-κB signaling pathway, are involved in delayed-type allergic responses such as contact hypersensitivity (CHS) (Klemm et al. 2006; Nishida et al. 2009). Mast cell-derived TNF is required for a maximum CHS response; it induces leukocyte infiltration at the inflammation site (Biedermann et al. 2000), enhances the elongation of cutaneous nerves (Kakurai et al. 2006), and enhances the dendritic cell migration to draining lymph nodes (Suto et al. 2006). In this study, we revealed that treating mice with the LTCC antagonist verapamil inhibits CHS, which is a delayed-type immune response, without affecting passive cutaneous anaphylaxis (PCA), which is an immediate-type response. Consistent with these results, we showed that verapamil treatment inhibits the FcεRI-mediated activation of NF-κB DNA-binding activity and cytokine gene induction, but not calcium elevation or degranulation in BMMCs. Thus, the inhibitory effect of verapamil on CHS might depend at least partly on the reduction of mast cell-derived cytokine production and be independent of histamine and other mediators.

In future studies, knocking out the LTCCα_{1D}-subunit in mice will further clarify Zn wave in vivo roles in allergic and other immune responses.

5.5 Perspective

We have discussed new insights into the relevance of Zn, its channels, and its transporters in immune cell responses, particularly focusing on the role of Zn as a signaling molecule. Our studies and those of other researchers indicate that Zn signals affect a variety of immune-signaling pathways to produce biological outputs. Thus, we propose that Zn functions as a signaling molecule (Fukada et al. 2011; Hirano et al. 2008; Murakami and Hirano 2008; Nishida et al. 2011). Many questions about the role of Zn in signaling remain to be answered. We do not yet know the other targets, the biological significance in vivo, or the regulatory mechanisms of Zn signaling, particularly in the Zn wave. As with calcium, it is likely that Zn is an important intracellular signaling molecule in various systems including immune system. We hope that recognition of the importance of Zn signaling and Zn biology will open new avenues for future research.

References

Altan-Bonnet G, Germain RN (2005) Modeling T cell antigen discrimination based on feedback control of digital ERK responses. PLoS Biol 3:e356

Andersson DA, Gentry C, Moss S, Bevan S (2009) Clioquinol and pyrithione activate TRPA1 by increasing intracellular Zn^{2+}. Proc Natl Acad Sci USA 106:8374–8379

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

Andrews GK (2001) Cellular zinc sensors: MTF-1 regulation of gene expression. Biometals 14:223–237

Assaf SY, Chung SH (1984) Release of endogenous Zn^{2+} from brain tissue during activity. Nature (Lond) 308:734–736

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

Berthet J, Rall TW, Sutherland EW (1957) The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J Biol Chem 224:463–475

Besser L, Chorin E, Sekler I, Silverman WF, Atkin S, Russell JT, Hershfinkel M (2009) Synaptically released zinc triggers metabotropic signaling via a zinc-sensing receptor in the hippocampus. J Neurosci 29:2890–2901

Bichet D, Cornet V, Geib S, Carlier E, Volsen S, Hoshi T, Mori Y, De Waard M (2000) The I-II loop of the Ca^{2+} channel alpha1 subunit contains an endoplasmic reticulum retention signal antagonized by the beta subunit. Neuron 25:177–190

Biedermann T, Kneilling M, Mailhammer R, Maier K, Sander CA, et al. (2000) Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. J Exp Med 192: 1441–1452

Brautigan DL, Bornstein P, Gallis B (1981) Phosphotyrosyl-protein phosphatase. Specific inhibition by Zn. J Biol Chem 256:6519–6522

Brazil DP, Hemmings BA (2001) Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci 26:657–664

Catterall WA (2000) Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol 16:521–555
Christine CW, Choi DW (1990) Effect of zinc on NMDA receptor-mediated channel currents in cortical neurons. J Neurosci 10:108–116
Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD (1999) Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. Proc Natl Acad Sci USA 96:1716–1721
Colvin RA, Fontaine CP, Laskowski M, Thomas D (2003) $Zn2^{+}$ transporters and Zn^{2+} homeostasis in neurons. Eur J Pharmacol 479:171–185
Corbalan-Garcia S, Gomez-Fernandez JC (2006) Protein kinase C regulatory domains: the art of decoding many different signals in membranes. Biochim Biophys Acta 1761:633–654
Cornet V, Bichet D, Sandoz G, Marty I, Brocard J, Bourinet E, Mori Y, Villaz M, De Waard M (2002) Multiple determinants in voltage-dependent P/Q calcium channels control their retention in the endoplasmic reticulum. Eur J Neurosci 16:883–895
Dal Porto JM, Gauld SB, Merrell KT, Mills D, Pugh-Bernard AE, Cambier J (2004) B cell antigen receptor signaling 101. Mol Immunol 41:599–613
Fernandes G, Nair M, Onoe K, Tanaka T, Floyd R, Good RA (1979) Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc Natl Acad Sci USA 76:457–461
Fraker PJ, Caruso R, Kierszenbaum F (1982) Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with *Trypanosoma cruzi*. J Nutr 112:1224–1229
Frederickson CJ, Bush AI (2001) Synaptically released zinc: physiological functions and pathological effects. Biometals 14:353–366
Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449–462
Fukada T, Kambe T (2011) Molecular and genetic features of zinc transporters in physiology and pathogenesis. Metallomics 3:662–674
Fukada T, Civic N, Furuichi T, Shimoda S, Mishima K, Higashiyama H, Idaira Y, Asada Y, Kitamura H, Yamasaki S et al (2008) The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. PLoS One 3:e3642
Fukada T, Yamasaki S, Nishida K, Murakami M, Hirano T (2011) Zinc homeostasis and signaling in health and diseases: zinc signaling. J Biol Inorg Chem 16:1123–1134
Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. Nature (Lond) 454:445–454
Gomperts BD, Tatham PER, Kramer IM (2002) Signal transduction. Academic, San Diego
Goode BL, Drubin DG, Barnes G (2000) Functional cooperation between the microtubule and actin cytoskeletons. Curr Opin Cell Biol 12:63–71
Gyulkhandanyan AV, Lee SC, Bikopoulos G, Dai F, Wheeler MB (2006) The Zn^{2+}-transporting pathways in pancreatic beta-cells: a role for the L-type voltage-gated Ca^{2+} channel. J Biol Chem 281:9361–9372
Haase H, Maret W (2005) Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants. Biometals 18:333–338
Haase H, Rink L (2009) Functional significance of zinc-related signaling pathways in immune cells. Annu Rev Nutr 29:133–152
Haase H, Hebel S, Engelhardt G, Rink L (2006) Flow cytometric measurement of labile zinc in peripheral blood mononuclear cells. Anal Biochem 352:222–230
Haase H, Ober-Blobaum JL, Engelhardt G, Hebel S, Heit A, Heine H, Rink L (2008) Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J Immunol 181:6491–6502
Harwood NE, Batista FD (2008) New insights into the early molecular events underlying B cell activation. Immunity 28:609–619
Harwood NE, Batista FD (2010) Early events in B cell activation. Annu Rev Immunol 28:185–210

Hashimoto A, Okada H, Jiang A, Kurosaki M, Greenberg S, Clark EA, Kurosaki T (1998) Involvement of guanosine triphosphatases and phospholipase C-gamma2 in extracellular signal-regulated kinase, c-Jun NH_2-terminal kinase, and p38 mitogen-activated protein kinase activation by the B cell antigen receptor. J Exp Med 188:1287–1295

Hershfinkel M, Moran A, Grossman N, Sekler I (2001) A zinc-sensing receptor triggers the release of intracellular Ca^{2+} and regulates ion transport. Proc Natl Acad Sci USA 98:11749–11754

Hirano T, Murakami M, Fukada T, Nishida K, Yamasaki S, Suzuki T (2008) Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. Adv Immunol 97:149–176

Hirzel K, Muller U, Latal AT, Hulsmann S, Grudzinska J, Seeliger MW, Betz H, Laube B (2006) Hyperekplexia phenotype of glycine receptor alpha1 subunit mutant mice identifies Zn(2+) as an essential endogenous modulator of glycinergic neurotransmission. Neuron 52:679–690

Ho LH, Ruffin RE, Murgia C, Li L, Krilis SA, Zalewski PD (2004) Labile zinc and zinc transporter ZnT4 in mast cell granules: role in regulation of caspase activation and NF-kappaB translocation. J Immunol 172:7750–7760

Hojyo S, Fukada T, Shimoda S, Ohashi W, Bin BH, Koseki H, Hirano T (2011) The zinc transporter SLC39A14/ZIP14 controls G-protein coupled receptor-mediated signaling required for systemic growth. PLoS One 6:e18059

Holst B, Egerod KL, Jin C, Petersen PS, Ostergaard MV, Hald J, Sprinkel AM, Storling J, Mandrup-Poulsen T, Holst JJ et al (2009) G protein-coupled receptor 39 deficiency is associated with pancreatic islet dysfunction. Endocrinology 150:2577–2585

Hosie AM, Dunne EL, Harvey RJ, Smart TG (2003) Zinc-mediated inhibition of GABA (A) receptors: discrete binding sites underlie subtype specificity. Nat Neurosci 6:362–369

Hou S, Vigeland LE, Zhang G, Xu R, Li M, Heinemann SH, Hoshi T (2010) Zn^{2+} activates large conductance Ca^{2+}-activated K^+ channel via an intracellular domain. J Biol Chem 285:6434–6442

Hu H, Bandell M, Petrus MJ, Zhu MX, Patapoutian A (2009) Zinc activates damage-sensing TRPA1 ion channels. Nat Chem Biol 5:183–190

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

Ibs KH, Rink L (2003) Zinc-altered immune function. J Nutr 133:1452S–1456S

Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, Sato E, Kitamura H, Nishida K, Hirano T (2006) Zinc is required for Fc-epsilon-RI-mediated mast cell activation. J Immunol 177:1296–1305

Kakurai M, Monteforte R, Suto H, Tsai M, Nakae S, Galli SJ (2006) Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. Am J Pathol 169:1713–1721

Kambe T, Yamaguchi-Iwai Y, Sasaki R, Nagao M (2004) Overview of mammalian zinc transporters. Cell Mol Life Sci 61:49–68

Kawakami T, Ando T, Kimura M, Wilson BS, Kawakami Y (2009) Mast cells in atopic dermatitis. Curr Opin Immunol 21:666–678

Keen CL, Gershwin ME (1990) Zinc deficiency and immune function. Annu Rev Nutr 10:415–431

Kikuchi K (2010) Design, synthesis and biological application of chemical probes for bio-imaging. Chem Soc Rev 39:2048–2053

Kitamura H, Morikawa H, Kamon H, Iguchi M, Hojyo S, Fukada T, Yamashita S, Kaisho T, Akira S, Murakami M, Hirano T (2006) Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. Nat Immunol 7:971–977

Klemm S, Gutermuth J, Hultner L, Sparwasser T, Behrendt H, Peschel C, Mak TW, Jakob T, Ruland J (2006) The Bcl10-Malt1 complex segregates Fc epsilon RI-mediated nuclear factor kappa B activation and cytokine production from mast cell degranulation. J Exp Med 203:337–347

Kurosaki T (2011) Regulation of BCR signaling. Mol Immunol 48:1287–1291

Lee JY, Cole TB, Palmiter RD, Suh SW, Koh JY (2002) Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. Proc Natl Acad Sci USA 99:7705–7710

Legendre P, Westbrook GL (1990) The inhibition of single *N*-methyl-D-aspartate-activated channels by zinc ions on cultured rat neurones. J Physiol 429:429–449

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

Levy S, Beharier O, Etzion Y, Mor M, Buzaglo L, Shaltiel L, Gheber LA, Kahn J, Muslin AJ, Katz A et al (2009) Molecular basis for zinc transporter 1 action as an endogenous inhibitor of L-type calcium channels. J Biol Chem 284:32434–32443

Li Y, Hough CJ, Suh SW, Sarvey JM, Frederickson CJ (2001) Rapid translocation of Zn(2+) from presynaptic terminals into postsynaptic hippocampal neurons after physiological stimulation. J Neurophysiol 86:2597–2604

Lichten LA, Cousins RJ (2009) Mammalian zinc transporters: nutritional and physiologic regulation. Annu Rev Nutr 29:153–176

Lopantsev V, Wenzel HJ, Cole TB, Palmiter RD, Schwartzkroin PA (2003) Lack of vesicular zinc in mossy fibers does not affect synaptic excitability of CA3 pyramidal cells in zinc transporter 3 knockout mice. Neuroscience 116:237–248

Lu YM, Taverna FA, Tu R, Ackerley CA, Wang YT, Roder J (2000) Endogenous Zn(2+) is required for the induction of long-term potentiation at rat hippocampal mossy fiber-CA3 synapses. Synapse 38:187–197

Mayer ML, Vyklicky L Jr (1989) The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. J Physiol 415:351–365

Metz M, Grimbaldeston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ (2007) Mast cells in the promotion and limitation of chronic inflammation. Immunol Rev 217:304–328

Murakami M, Hirano T (2008) Intracellular zinc homeostasis and zinc signaling. Cancer Sci 99:1515–1522

Murakami K, Whiteley MK, Routtenberg A (1987) Regulation of protein kinase C activity by cooperative interaction of Zn^{2+} and Ca^{2+}. J Biol Chem 262:13902–13906

Nechushtan H, Leitges M, Cohen C, Kay G, Razin E (2000) Inhibition of degranulation and interleukin-6 production in mast cells derived from mice deficient in protein kinase C-beta. Blood 95:1752–1757

Nishida K, Yamasaki S, Ito Y, Kabu K, Hattori K, Tezuka T, Nishizumi H, Kitamura D, Goitsuka R, Geha RS et al (2005) FcεRI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. J Cell Biol 170:115–126

Nishida K, Hasegawa A, Nakae S, Oboki K, Saito H, Yamasaki S, Hirano T (2009) Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. J Exp Med 206:1351–1364

Nishida K, Fukada T, Yamasaki S, Murakami M, Hirano T (2011) Zinc in allergy, autoimmune, and hard and connective tissue diseases. IOS Press, Netherlands

Oancea E, Teruel MN, Quest AF, Meyer T (1998) Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. J Cell Biol 140:485–498

Palacios EH, Weiss A (2004) Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene 23:7990–8000

Palmiter RD (2004) Protection against zinc toxicity by metallothionein and zinc transporter 1. Proc Natl Acad Sci USA 101:4918–4923

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

Perry DK, Smyth MJ, Stennicke HR, Salvesen GS, Duriez P, Poirier GG, Hannun YA (1997) Zinc is a potent inhibitor of the apoptotic protease, caspase-3. A novel target for zinc in the inhibition of apoptosis. J Biol Chem 272:18530–18533

Prasad AS (1991) Discovery of human zinc deficiency and studies in an experimental human model. Am J Clin Nutr 53:403–412

Prasad AS (1995) Zinc: an overview. Nutrition 11:93–99

Prost AL, Bloc A, Hussy N, Derand R, Vivaudou M (2004) Zinc is both an intracellular and extracellular regulator of KATP channel function. J Physiol 559:157–167

Qian J, Noebels JL (2005) Visualization of transmitter release with zinc fluorescence detection at the mouse hippocampal mossy fibre synapse. J Physiol 566:747–758

Ramadan O, Qu Y, Wadgaonkar R, Baroudi G, Karnabi E, Chahine M, Boutjdir M (2009) Phosphorylation of the consensus sites of protein kinase A on alpha1D L-type calcium channel. J Biol Chem 284:5042–5049

Riccioni G, D'Orazio N (2005) The role of selenium, zinc and antioxidant vitamin supplementation in the treatment of bronchial asthma: adjuvant therapy or not? Expert Opin Invest Drugs 14:1145–1155

Richter M, Bonneau R, Girard MA, Beaulieu C, Larivee P (2003a) Zinc status modulates bronchopulmonary eosinophil infiltration in a murine model of allergic inflammation. Chest 123:446S

Richter M, Cantin AM, Beaulieu C, Cloutier A, Larivee P (2003b) Zinc chelators inhibit eotaxin, RANTES, and MCP-1 production in stimulated human airway epithelium and fibroblasts. Am J Physiol Lung Cell Mol Physiol 285:L719–L729

Rink L, Gabriel P (2000) Zinc and the immune system. Proc Nutr Soc 59:541–552

Schnapp BJ (2003) Trafficking of signaling modules by kinesin motors. J Cell Sci 116:2125–2135

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

Sensi SL, Paoletti P, Bush AI, Sekler I (2009) Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci 10:780–791

Sharir H, Zinger A, Nevo A, Sekler I, Hershfinkel M (2010) Zinc released from injured cells is acting via the Zn^{2+}-sensing receptor, ZnR, to trigger signaling leading to epithelial repair. J Biol Chem 285:26097–26106

Shumilina E, Lam RS, Wolbing F, Matzner N, Zemtsova IM, Sobiesiak M, Mahmud H, Sausbier U, Biedermann T, Ruth P et al (2008) Blunted IgE-mediated activation of mast cells in mice lacking the Ca^{2+}-activated K^+ channel KCa3.1. J Immunol 180:8040–8047

Smart TG, Hosie AM, Miller PS (2004) Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. Neuroscientist 10:432–442

Smith AJ, Pfeiffer JR, Zhang J, Martinez AM, Griffiths GM, Wilson BS (2003) Microtubule-dependent transport of secretory vesicles in RBL-2H3 cells. Traffic 4:302–312

Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ (2006) Mast cell-associated TNF promotes dendritic cell migration. J Immunol 176:4102–4112

Suzuki T, Ishihara K, Migaki H, Matsuura W, Kohda A, Okumura K, Nagao M, Yamaguchi-Iwai Y, Kambe T (2005a) Zinc transporters, ZnT5 and ZnT7, are required for the activation of alkaline phosphatases, zinc-requiring enzymes that are glycosylphosphatidylinositol-anchored to the cytoplasmic membrane. J Biol Chem 280:637–643

Suzuki T, Ishihara K, Migaki H, Nagao M, Yamaguchi-Iwai Y, Kambe T (2005b) Two different zinc transport complexes of cation diffusion facilitator proteins localized in the secretory pathway operate to activate alkaline phosphatases in vertebrate cells. J Biol Chem 280:30956–30962

Tamaki M, Fujitani Y, Hara A, Uchida T, Tamura Y, Takeno K, Kawaguchi M, Watanabe T, Ogihara T, Fukunaka A et al (2013) The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. J Clin Invest 123:4513–4524

Taniguchi M, Fukunaka A, Hagihara M, Watanabe K, Kamino S, Kambe T, Enomoto S, Hiromura M (2013) Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in B-cell receptor signaling pathway in DT40 cells. PLoS One 8:e58022

Taylor KM, Vichova P, Jordan N, Hiscox S, Hendley R, Nicholson RI (2008) ZIP7-mediated intracellular zinc transport contributes to aberrant growth factor signaling in antihormone-resistant breast cancer cells. Endocrinology 149:4912–4920

Taylor KM, Hiscox S, Nicholson RI, Hogstrand C, Kille P (2012) Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. Sci Signal 5:ra11

Telford WG, Fraker PJ (1995) Preferential induction of apoptosis in mouse CD4 + CD8+ alpha beta TCRloCD3 epsilon lo thymocytes by zinc. J Cell Physiol 164:259–270

Thambiayya K, Wasserloos KJ, Huang Z, Kagan VE, St. Croix CM, Pitt BR (2011) 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 300:L624–L632

Torres-Alanis O, Garza-Ocanas L, Pineyro-Lopez A (1995) Evaluation of urinary mercury excretion after administration of 2,3-dimercapto-1-propane sulfonic acid to occupationally exposed men. J Toxicol Clin Toxicol 33:717–720

Truong-Tran AQ, Ruffin RE, Zalewski PD (2000) Visualization of labile zinc and its role in apoptosis of primary airway epithelial cells and cell lines. Am J Physiol Lung Cell Mol Physiol 279:L1172–L1183

Ueno S, Tsukamoto M, Hirano T, Kikuchi K, Yamada MK, Nishiyama N, Nagano T, Matsuki N, Ikegaya Y (2002) Mossy fiber Zn^{2+} spillover modulates heterosynaptic *N*-methyl-D-aspartate receptor activity in hippocampal CA3 circuits. J Cell Biol 158:215–220

Vallee BL (1995) The function of metallothionein. Neurochem Int 27:23–33

Vallee BL, Auld DS (1993) Cocatalytic zinc motifs in enzyme catalysis. Proc Natl Acad Sci USA 90:2715–2718

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

Vennekens R, Olausson J, Meissner M, Bloch W, Mathar I, Philipp SE, Schmitz F, Weissgerber P, Nilius B, Flockerzi V, Freichel M (2007) Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. Nat Immunol 8:312–320

Vig M, Kinet JP (2009) Calcium signaling in immune cells. Nat Immunol 10:21–27

Vogt K, Mellor J, Tong G, Nicoll R (2000) The actions of synaptically released zinc at hippocampal mossy fiber synapses. Neuron 26:187–196

Weiss JH, Sensi SL (2000) Ca^{2+}-Zn^{2+} permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration. Trends Neurosci 23:365–371

Xie X, Smart TG (1994) Modulation of long-term potentiation in rat hippocampal pyramidal neurons by zinc. Pflugers Arch 427:481–486

Xu TF, Wang XL, Yang JZ, Hu XY, Wu WF, Guo L, Kang LD, Zhang LY (2009) Overexpression of Zip-2 mRNA in the leukocytes of asthmatic infants. Pediatr Pulmonol 44:763–767

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

Yamasaki S, Hasegawa A, Hojyo S, Ohashi W, Fukada T, Nishida K, Hirano T (2012) A novel role of the L-type calcium channel alpha1D subunit as a gatekeeper for intracellular zinc signaling: zinc wave. PLoS One 7:e39654

Yamashita S, Miyagi C, Fukada T, Kagara N, Che YS, Hirano T (2004) Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer. Nature (Lond) 429:298–302

Yasuda S, Miyazaki T, Munechika K, Yamashita M, Ikeda Y, Kamizono A (2007) Isolation of Zn^{2+} as an endogenous agonist of GPR39 from fetal bovine serum. J Recept Signal Transduct Res 27:235–246

Yu M, Lee WW, Tomar D, Pryshchep S, Czesnikiewicz-Guzik M, Lamar DL, Li G, Singh K, Tian L, Weyand CM, Goronzy JJ (2011) Regulation of T cell receptor signaling by activation-induced zinc influx. J Exp Med 208:775–785

Zalewski PD, Truong-Tran AQ, Grosser D, Jayaram L, Murgia C, Ruffin RE (2005) Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review. Pharmacol Ther 105:127–149