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Techniques for illuminating the cell biology of zinc

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Abstract

Zinc (Zn^{2+}) is an essential micronutrient that is required for a wide variety of cellular processes. Tools and methods have been instrumental in revealing the myriad roles of Zn^{2+} in cells. This review highlights recent developments fluorescent sensors to measure the labile Zn^{2+} pool, chelators to manipulate Zn^{2+} availability, and fluorescent tools and proteomics approaches for monitoring Zn^{2+}-binding proteins in cells. Finally, we close with some highlights on the role of Zn^{2+} in regulating cell function and in cell signaling.

Keywords: Zinc; Tools Methods; Fluorescent sensors; Proteomics; Chelators.

Introduction

Zn^{2+} is required by thousands of cellular proteins where it has structural, catalytic and regulatory functions . Zn^{2+} regulates a wide-range of molecular processes that can profoundly affect cellular and organismal biology [. The total concentration of Zn^{2+} within mammalian cells is on the order of hundreds of micromolar, while readily exchangeable or labile Zn^{2+} is maintained on the order of hundreds of picomolar. Multiple studies have demonstrated that in a wide range of mammalian cell types cytosolic Zn^{2+} levels are near 100 pM and the labile Zn^{2+} pool can fluctuate in response to extracellular stimuli.Tools and techniques that are capable of differentiating the labile and bound Zn^{2+} pools, manipulating Zn^{2+} levels in media and in cells, and proteomic approaches for profiling the Zn^{2+} proteome are critical to our understanding of Zn^{2+} biology [1].

Illuminating Zn^{2+} in living cells with fluorescent sensors

In contrast with elemental mapping techniques which can provide valuable information about total Zn^{2+} distribution in cells fluorescent Zn^{2+} sensors can specifically report on the labile Zn^{2+} pool. Fluorescent Zn^{2+} sensors come in two main flavors: genetically encoded and small-molecule sensors. Both classes contain a metal-binding group and at least one fluorophore that absorbs and emits light in the visible region of the electromagnetic spectrum. Zn^{2+} binding results in a change in the overall structure or electronic configuration of the sensor, resulting in a change in fluorescence that can be measured using a fluorescence microscope.

When selecting a Zn^{2+} sensor, one must consider characteristics inherent to the fluorophore, such as absorption and emission properties, photostability, pH-sensitivity and brightness, as well as metal-binding properties, like selectivity, kinetics and affinity. Fluorescent sensors are most sensitive to changes in Zn^{2+} concentrations that are near their apparent dissociation constant for Zn^{2+} binding (K_d'). The K_d' is defined as the labile Zn^{2+} concentration at which the sensor is half-saturated and can be determined by performing an *in vitro* or *in situ* Zn^{2+} titration. Another important characteristic of fluorescent sensors is the dynamic range, which is determined by the fluorescence signal in the Zn^{2+}-bound state *versus* the fluorescence signal in the Zn^{2+}-unbound state. In general, a sensor with a high dynamic range can provide both sensitivity and accuracy for estimating Zn^{2+} levels. Ultimately, selecting a sensor will depend on the intended application. For instance, genetically encoded Zn^{2+} sensors can be selectively targeted to subcellular compartments as a means to measure Zn^{2+} within organelles or detect Zn^{2+} release at the cell surface the *in situ* dynamic range, K_d' and targeted cellular compartments of the current repertoire of genetically encoded Zn^{2+} sensors. In contrast, small-molecule sensors are difficult to direct to a particular compartment, but they often offer a large dynamic range making them particularly sensitive to Zn^{2+} dynamics. Hybrid, or chemigenetic, sensors are a promising new set of tools that leverage small-molecule Zn^{2+} sensors and take advantage of a genetically encoded component to target organelles. In this review, we cover recent advances in the development and application of fluorescent Zn^{2+} sensors. We also refer readers to excellent reviews that cover earlier work in the field [3].

Applications of tools to probe cell biology of Zn^{2+}

Zn^{2+} in cellular regulation

It is widely known that Zn^{2+} is necessary for cell proliferation, as Zn^{2+} deficient cells fail to divide and proliferate and one of the symptoms of Zn^{2+} deficiency is stunted growth. However, the molecular mechanisms of how Zn^{2+} deficiency leads to cell cycle arrest have remained elusive. Recently, new tools to study both Zn^{2+} and the cell cycle have begun to provide insight into the role of Zn^{2+}. Cell cycle studies typically rely on serum starvation of cells to synchronize the cell cycle phases across a population. While serum starvation removes essential growth factors such as mitogens, it also removes essential vitamins and minerals, including Zn^{2+}. Furthermore, cell synchronization can induce stress response pathways, making it difficult to correlate findings to naturally cycling cells. Advances in fluorescent reporters, high throughput microscopy, and quantitative image analysis have made it possible to monitor cell cycle phases in naturally cycling cells to characterize cells' temporary exit from the cell cycle, termed quiescence [4].

In an asynchronously-dividing population of cells, Lo and colleagues were able to track cells throughout the cell cycle and determine which aspects of the cell cycle are dependent on Zn^{2+}. Cells were grown in media with chelex-treated serum to create a minimal media

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Zn^{2+} condition (1.46 μM Zn^{2+}, measured by ICP-MS), and experimental conditions were either supplemented with 30 μM Zn^{2+} (Zn^{2+} replete) or further Zn^{2+}-restricted by the addition of 2–3 μM TPA. The metal in cells was then quantified using the genetically encoded sensor ZapCV2, which demonstrated that media Zn^{2+} and TPA manipulation result in changes in labile cellular Zn^{2+}, and that these manipulations are not toxic to cells for the duration of the experiment. The Zn^{2+} deficient condition was found to either induce cellular quiescence or cell cycle stall in S-phase, with insufficient DNA replication. The cellular response to Zn^{2+} deficiency was shown to be dependent on when in the cell cycle Zn^{2+} deficiency was induced. Interestingly, DNA damage was increased in the Zn^{2+}-deficient cells that continued through the cell cycle, but not their quiescent neighbors, suggesting that while Zn^{2+} is necessary for DNA replication and repair, its role in the proliferation/quiescence decision is independent of DNA damage. Furthermore, resupply of Zn^{2+} promotes cell cycle re-entry, but the mechanism by which proteins and signaling pathways mediate cell cycle re-entry has not been identified.

Zn^{2+} in cell signaling

Another major push in the field of Zn^{2+} biology is understanding systems in which Zn^{2+} can act as a cellular signal. A variety of cell systems have been shown to exhibit dynamic Zn^{2+} behavior, including neurons and oocytes. It has long been known that specific regions of the brain are Zn^{2+}-rich and that the Zn^{2+} transporter, ZnT3, imports Zn^{2+} into synaptic vesicles. Along with ZnT3 knockout mice, the extracellular Zn^{2+} chelator ZX1 has led to insights about the role of vesicular Zn^{2+} in neurons. Zn^{2+} has been shown to modulate signaling through neurotransmitter receptors, including the inhibition of NMDA glutamate receptors. New electrophysiology studies show that synaptically-released Zn^{2+} from hippocampal mossy fiber neurons contributes to long term potentiation, or strengthening of neuronal synaptic connections Recently, Sanford and colleagues quantified Zn^{2+} dynamics upon stimulation by KCl in cultured hippocampal neurons and demonstrated that over 900 genes exhibit changes in expression in a Zn^{2+}-dependent manner in response to subnanomolar fluctuations of Zn^{2+}. Many of these transcriptional changes involve synaptic plasticity pathways Furthermore, *in vivo* animal studies have demonstrated that Zn^{2+} may play a role in fear conditioning, long-term and spatial memory, and audition. These *in vivo* phenotypes are subtle, suggesting that synaptic Zn^{2+} may play more of a role in fine-tuning neuronal connections or that there are compensatory mechanisms during development that mask synaptic Zn^{2+} deficiency phenotypes.

While it has long been recognized that Zn^{2+} is concentrated in mossy fiber neurons in the hippocampus and released with synaptic activity through studies in brain slices and animals, cellular models of Zn^{2+} dynamics are far less clear. In dissociated hippocampal neuron culture, stimulation with glutamate/glycine or KCl has been shown to increase intracellular Zn^{2+}, and this Zn^{2+} signal has important downstream signaling consequences. While it was routinely assumed that this intracellular Zn^{2+} derived from synaptic release, more recent studies suggest that this Zn^{2+} may arise from an intracellular source. In particular, it was suggested that neuronal acidification upon glutamate treatment was responsible for Zn^{2+} mobilization. Sanford and Palmer recently quantified Zn^{2+}, Ca^{2+}, and pH changes using a series of fluorescent sensors during stimulation of dissociated hippocampal neurons in culture. Both KCl and glutamate stimulation led to increases in cytosolic Zn^{2+}, and the signal was comparable in the presence of ZX1, suggesting that in dissociated neuron culture Zn^{2+} was released from intracellular stores. Although the pH decreased upon neuronal stimulation, the magnitude and timing of the changes in pH did not correlate with the magnitude and timing of Zn^{2+} changes, suggesting that Zn^{2+} release from intracellular stores might be due to Ca^{2+} dynamics or reactive oxygen species (ROS) production [5].

Another cellular system that experiences Zn^{2+} signals is the developing oocyte. Recently, Zn^{2+} accumulation during oocyte maturation and release at fertilization were rigorously quantified to better understand the source of the "Zn^{2+} spark" at fertilization. Que and colleagues, used the fluorescent Zn^{2+} probe ZincBY-1, elemental mapping, and extracellular FluoZin-3 Zn^{2+} dye to show that oocytes contain thousands of vesicles loaded with approximately 1 million Zn^{2+} ions each, and that these vesicles undergo exocytosis when the oocyte becomes fertilized, resulting in the extracellular Zn^{2+} spark. Subsequent research demonstrated that the Zn^{2+} released from oocytes upon fertilization is linked to the hardening of the zona pellucida, the oocyte's glycoprotein extracellular matrix, which prevents subsequent fertilizations and maintains the viability of the newly formed zygote . Furthermore, larger Zn^{2+} sparks have been shown to be indicative of future embryo quality, suggesting that Zn^{2+} could potentially be used as a biomarker for fertility treatments. These recent discoveries in oocyte biology suggest that Zn^{2+} regulation and dynamics can play multiple roles in cell development and physiology.

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Conflict of Interest:

None

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