Biochimica et Biophysica Acta 1823 (2012) 1553–1567

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The zinc homeostasis network of land plants☆

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ARTICLE INFO

Article history:
Received 29 February 2012
Received in revised form 8 May 2012
Accepted 13 May 2012
Available online 22 May 2012

Keywords:
Zn
Fe
Metal
Hyperaccumulator plant
Arabidopsis
Metal regulation

ABSTRACT

The use of the essential element zinc (Zn) in the biochemistry of land plants is widespread, and thus comparable to that in other eukaryotes. Plants have evolved the ability to adjust to vast fluctuations in external Zn supply, and they can store considerable amounts of Zn inside cell vacuoles. Moreover, among plants there is overwhelming, but yet little explored, natural genetic diversity that phenotypically affects Zn homeostasis. This results in the ability of specific races or species to thrive in different soils ranging from extremely Zn-deficient to highly Zn-polluted. Zn homeostasis is maintained by a tightly regulated network of low-molecular-weight ligands, membrane transport and Zn-binding proteins, as well as regulators. Here we review Zn homeostasis of land plants largely based on the model plant *Arabidopsis thaliana*, for which our molecular understanding is most developed at present. There is some evidence for substantial conservation of Zn homeostasis networks among land pants, and this review can serve as a reference for future comparisons. Major progress has recently been made in our understanding of the regulation of transcriptional Zn deficiency responses and the role of the low-molecular-weight chelator nicotianamine in plant Zn homeostasis. Moreover, we have begun to understand how iron (Fe) and Zn homeostasis interact as a consequence of the chemical similarity between their divalent cations and the lack of specificity of the major root iron uptake transporter IRT1. The molecular analysis of Zn-hyperaccumulating plants reveals how metal homeostasis networks can be effectively modified. These insights are important for sustainable bio-fortification approaches. This article is part of a Special Issue entitled: Cell Biology of Metals.

1. Introduction

Zinc (Zn) is an essential element in all organisms. In its oxidized Zn(II)[1] form, which is the form of Zn found throughout biology, it acts as a catalytic or structural co-factor in a large number of enzymes and regulatory proteins [1]. Well-known examples in plants include the enzymes carbonic anhydrase and alcohol dehydrogenase, and the structural Zn-finger domains mediating DNA-binding of transcription factors and protein–protein interactions [2]. The use of a specific element in biological chemistry is a result of a complex interplay between various factors during evolution and under present-day environmental conditions. Among these, important factors are the bio-availability of the element for an organism, the efficacy and specificity of the element in fulfilling its various biochemical functions in comparison to elements that could be utilized alternatively, and constraints posed by the protein complement of the organism. Over the course of evolution, life has increasingly made use of Zn, with the Zn metalloproteome predicted to comprise around 5 to 6% of prokaryotic proteome and about 9% of the eukaryotic proteome [3].

Distinctive chemical properties make Zn a highly effective cofactor [4]. Among metals, Zn(II) is a strong and efficient Lewis acid catalyst and has very high binding affinity to a variety of ligands. Zn(II) is exceptionally flexible in the coordination geometries that it can adopt. Moreover, under biological conditions, Zn never undergoes changes in redox state so that it cannot directly participate in electron transfer reactions, for example in electron transport chains. When compared to transition metals that are able to accept or donate electrons themselves, such as Fe and Cu, the use of Zn is comparably safe in the proximity of sensitive macromolecules, in particular DNA, in the nucleus. The availability of Zn, which forms insoluble sulphides, has increased since the oxygenation of the Earth's atmosphere, and Zn concentrations are lower in aqueous environments than on land. This is probably the reason why the diatom, *Thalassiosira weissflogii* has evolved the ability to functionally replace Zn in carbonic anhydrase by Cadmium (Cd) when Zn is scarce, manifesting a Cd requirement that is unique in biology based on our present state of knowledge [5]. There is a pressing need for conclusive experimental approaches to determine the Zn metalloproteome in a number of organisms in order to experimentally validate *in silico* predictions based on the presence of amino acid sequence motifs.

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[1] Zn^{2+} refers either to the "free" hydrated cation in solution or the cation alone. Elsewhere, Zn or Zn(II) are used synonymously, taking into account that the predominant proportion of Zn(II) is present either in complexed form bound to inorganic or organic ligands (e.g., inside cells) or in the form of insoluble salts (e.g., in soil).

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doi:10.1016/j.bbamcr.2012.05.016

Not surprisingly, as a consequence of the relatively abundant use of Zn in biological chemistry, Zn deficiency is a widespread condition. The WHO estimates that 31% of the world's population is at risk of Zn deficiency [6]. As there are no reliable biomarkers for Zn deficiency, its prevalence has long been underrated – different from Fe deficiency. Zn deficiency has a severe impact on public health and infant mortality, causing growth, cognitive and immune impairment, including enhanced susceptibility to diarrhea. In general, Zn deficiency is particularly widespread among the elderly, and it affects children most severely in regions of the world where the population relies primarily on cereal diets [7], which are poor in bioavailable Zn. Moreover, grain Zn concentrations have received little attention in breeding programs to date and have steadily decreased in cultivated wheat varieties since the green revolution [8]. In recent years, pronounced efforts have been made to increase Zn content and availability in staple crops (e.g., HarvestPlus, http://www.harvestplus.org/). These efforts are still at an early stage because the physiological and developmental processes controlling Zn accumulation in cereal grains, as well as the genes governing them, remain poorly understood [9]. Nevertheless, systematic efforts to screen natural genetic diversity for the purpose of introgressing identified target alleles are underway, and some pilot studies involving transgenic plants have yielded promising results [10,11] (see below).

A significant proportion of the Earth's arable land is considered Zn-deficient [12,13]. It is of paramount importance to take into account that Zn deficiency does not merely reduce crop quality and nutritive value. Micronutrient acquisition and micronutrient use efficiency are traits that critically affect crop yield [14,15] (see Zn deficiency symptoms below). This important fact has not received sufficient attention to date, given that there is a need to substantially increase crop yields in order to accommodate foreseeable population growth, increasing need for renewable non-food resources and the accelerating decrease in the area of arable land.

Although total Zn concentrations in eukaryotic cells are of the order of 100 μM, the internal concentration of free Zn is generally below the nanomolar range and, in *Escherichia coli*, below the femtomolar range [16]. Tightly controlling the concentrations and chemical speciation of intracellular Zn is a necessity for all organisms, because the binding of Zn^{2+} to non-target sites would inevitably render these biologically non-functional. This has led to the evolution of a complex homeostatic network of Zn transporters, low-molecular-weight ligands and, although yet unknown in land plants, potentially also metallochaperone proteins that ensure the targeted delivery of the correct amounts of Zn to each apo-Zn protein, cellular compartment, cell, tissue and organ [17]. The concerted regulation of Zn homeostasis processes allows enhanced acquisition and redistribution of Zn, or storage and sequestration, in response to fluctuating environmental conditions and locally varying internal demands throughout the life cycle. How exactly this is accomplished remains poorly understood.

Various approaches have been developed in recent years with the aim of measuring cellular Zn concentrations *in vivo*. The Zn-fluorophore, Zinpyr-1, was used to image Zn within roots of *Arabidopsis thaliana*. As Zn-Zinpyr-1-dependent fluorescence is contingent upon the amount of Zn available for interaction with the fluorophore, comparisons of relative Zn levels between conditions and genotypes are possible [18]. However, the distribution of the fluorophore between and within cells cannot be fully controlled. The recent development of genetically encoded fluorescence resonance energy transfer-based ratiometric Zn sensors is very promising [19,20]. Using these sensors, dynamics of cytoplasmically available Zn concentrations were followed in mammalian cells. Estimates of resting cytoplasmic concentrations of 'free' Zn in mammalian cells between 5 pM and 1 nM were reported, with about 100-fold lower concentrations in ER and Golgi [19]. The targeting of sensors to specific intracellular compartments will help to experimentally address Zn concentrations at sub-cellular resolution. Cells control internal levels of Zn primarily by regulating transport processes that move Zn across membranes [21], with considerably less experimental evidence to date for the contribution of Zn binding. In most organisms, Zn is acquired either from the environment or from the diet by specific membrane transport proteins, sometimes operating in conjunction with chelators, which increase in abundance in response to Zn deficiency [22]. Work in yeast suggested that external Zn can change very rapidly, requiring the presence of proteins acting in Zn detoxification even in Zn-deficient cells [23]. In addition, transporters of divalent metal cations often exhibit broad substrate specificity, so that a deficiency in Cu, Fe or Mg may result in enhanced uptake and accumulation of toxic amounts of Zn. In these situations, Zn-specific transporters are needed in order to export excess Zn from the cytoplasm [24]. This has also been observed in plants [25]. In this review, we focus on the recent discoveries concerning Zn homeostasis in land plants, with a focus primarily on the model plant *A. thaliana* and closely related species.

2. Exceptionally large natural diversity in Zn homeostasis of land plants

Generally, plants exhibit Zn deficiency symptoms at shoot concentrations below a minimum of 15 to 20 mg Zn kg^{-1} dry biomass. These symptoms include reduced biomass production, poor floral fertility, leaf chlorosis, increased shoot branching and early senescence of older leaves [15,26] (Talke & Krämer, unpublished observations). Zn deficiency leads to increased production of reactive oxygen species resulting from lowered Cu/Zn superoxide dismutase (CuZn-SOD) activity, the inhibition of protein synthesis and increased Fe accumulation. This causes damage to membranes, membrane proteins, chlorophyll and enzymes, resulting in leaf chlorosis and the inhibition of photosynthesis and growth [27]. Some plants are able to grow on highly Zn-deficient soils, but little is known about the genetic basis of this ability. Zn micronutrient use efficiency in wheat cultivars is not correlated with elevated rates of either root Zn uptake or translocation to the shoot, but instead with the ability to maintain activities of the Zn-dependent enzymes Cu/Zn-SOD and carbonic anhydrase even upon cultivation under Zn-deficient conditions [14].

Human activities such as mining, industrial contamination, sewage sludge amendments and agriculture have contributed to the pollution of large areas of agricultural soils with Zn. In addition, rarely occurring so-called calamine soils are naturally rich in Zn. While Zn contamination and the resulting bio-accumulation hardly ever reach levels that are toxic to mammals, Zn toxicity is a major environmental concern in other organisms. Importantly, the occurrence of Zn in minerals is usually linked with the occurrence of the chemically similar, highly toxic carcinogen cadmium (Cd), and often also of lead (Pb). The predominant threat for human health is Cd contamination and, in particular, its progressive accumulation along the food chain. Decontamination of Cd-polluted sites currently involves the expensive excavation of contaminated soil and disposal. One potential solution to this problem is phytoremediation, which aims to exploit the trait of hyperaccumulation of Zn and other metals found in a small number of plants. Zn hyperaccumulators, of which fourteen taxa are presently known, grow naturally on metalliferous soils and accumulate $>1\%$ (w/w) Zn in dry leaf tissues upon growth at their natural site [28,29]. The best studied Zn hyperaccumulators, *Arabidopsis halleri* and *Noccaea* (formerly *Thlaspi*) *caerulescens*, are also hyperaccumulators of Cd, i.e., capable of leaf accumulation of $>0.01\%$ (w/w) Cd. Characteristic of Zn hyperaccumulators are dramatically elevated rates of root-to-shoot translocation of Zn and very high tolerance to Zn, especially in above-ground tissues. However, hyperaccumulators are generally low-biomass plants and unsuitable for large-scale phytoremediation. Understanding the molecular basis of