Functional Genomics and Gene Regulation in Biometals Research

Responses of plants to iron, zinc and copper deficiencies
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Abstract
Iron, copper and zinc are essential metals for cell metabolism. Plants have evolved different schemes to efficiently mobilize low-solubility nutrients such as metals from their environment and to transport them between organs. In this review we highlight the divergences and convergences of the iron, copper and zinc uptake, transport and homeostatic pathways.

Introduction
Transition metals are indispensable for life in all organisms. Because of their ability to donate and accept electrons, metals such as iron and copper are found as components of proteins that catalyse redox reactions. Zinc also provides a structural role in many transcription factors and is a cofactor of RNA polymerase, making it vital to cells. The same ability of these essential metal ions to transfer electrons can also make them toxic to cells when present in excess because they are directly involved in the formation of toxic reactive oxygen species (oxidative stress). To protect themselves, cells maintain metal levels in a very narrow range between deficiency and toxicity. To maintain adequate levels of metal ions in tight homoeostasis, all organisms, including bacteria, yeast, plants and humans, have evolved complex regulatory mechanisms of metal uptake, secretion and storage. Some of these mechanisms have been conserved through evolution. Plants can regulate their intracellular concentrations of metal ions in response to external stimuli, by modulating uptake, transport, storage and secretion rates. Some of the genes involved in copper, iron and zinc uptake in plants have been characterized, although our knowledge of how metal homoeostasis is regulated is only rudimentary. Genome-scale technologies are now providing powerful and exciting approaches for rapidly discerning, integrating and understanding molecular mechanisms that organisms use to adapt to their environment. A better understanding of metal metabolism in plants is a prerequisite for improving crop yield in metal-deficient soils and to allow nutritional improvement of crop plants to combat the widespread problem of dietary zinc and iron deficiencies.

Metal deficiencies in plants
Plants confronted with a nutritional deficiency, such as in copper, iron or zinc, change the expression of a series of genes and activate morphological changes, such as in root architecture. This leads to an increase in nutrient uptake and retention, and allows plants to explore more soil surface area to find nutrients. The up- and down-regulation of genes directing these events involve a series of molecular events that begin with the plant 'sensing' the deficiency and then transmitting the signal along transduction pathways and across the plant vascular system. Signals between the aerial parts of the plants, including the apical meristem, and the roots lead to the activation or inactivation of transcription factors that influence expression of specific genes. At the cellular level, post-transcriptional regulation may also occur. How plants detect and respond to deficiencies in copper, iron or zinc is still obscure. Plants have evolved different systems to efficiently obtain micronutrients available in the environment and keep them under tight homoeostasis. Physical properties, bio-availability, toxicity and plant requirements for copper, zinc and iron differ. Thus the mechanisms regulating the uptake and homoeostasis of each of these metals also differ. It is now becoming clear that proteins and molecules involved in their homoeostasis often derive from common ancestors.

Iron
Iron is one of the most abundant elements in the Earth's crust, yet in an oxygenated environment it

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is present in insoluble forms as Fe(III) hydroxides, oxides and phosphates, and free Fe(III) levels are far from adequate. Thus plants often face iron deficiency. Iron concentrations in cells typically range between 50 and 250 μg · g of dry weight⁻¹ [1]. Dicotyledonous plants such as Arabidopsis have developed mechanisms to facilitate iron uptake by increasing Fe(III) solubility through acidification of the rhizosphere and exudation of chelators such as organic acids. These plants increase reduction of Fe(III)-chelates using plasma-membrane ferric reductases to increase the level of Fe(II), the transported form of iron. [Grasses, unlike other plants, take up Fe(III) complexed to phytosiderophores, which are plant-derived metallophores.] In Arabidopsis, transport of Fe(II) occurs through members of the ZIP family of metal transporters [2] (IRT1, IRT2), Nramp-like transporters [3] and ferroportins, which are similar to the mammalian IREG1 gene involved in iron egress from intestinal enterocytes [4]. Iron is transported between organs in the plant via the phloem and the xylem where it is chelated to organic molecules such as citrate, nicotianamine and perhaps other compounds. In cells, excess iron is stored in a non-toxic form by incorporation into ferritin. However, plant ferritin is localized in the chloroplasts, not in the cytoplasm as in mammals, implying the need for iron-chloroplast transporters. Chloroplasts not only store iron but also catalyse the assembly of iron into haem using a plastid ferrochelatase [5], suggesting that trafficking of iron and haem in and out of chloroplasts is important in plant iron metabolism and homeostasis. For a recent detailed review on iron acquisition in plants see [6].

Copper

Among the transition metals, copper is the most toxic because of its high redox properties. Copper concentrations in the cells need to be maintained at low levels and thus typical plant copper concentrations range between 5 and 20 μg · g of dry weight⁻¹. The critical free copper activity in the nutrient media (below which copper deficiency occurs) ranges from 10⁻¹⁰ to 10⁻¹⁶ M. Plants usually find an ample supply of copper in soils, since typical soil solution concentrations range from 10⁻⁸ to 10⁻⁹ M, but plants may still need to solubilize and reduce the metal [1]. To date, no specific transporter involved in copper uptake from the environment has been characterized but there is evidence that copper is reduced by the iron-reductase system [7]. A transporter of the ZIP family of metal transporters that is involved in zinc uptake also has high affinity for copper, suggesting that some ZIP transporters may also transport copper into plant cells [8]. Intracellular transport, chelation and intercellular transport of copper may be important components of copper homeostasis in plants. A number of genes potentially involved in copper transport exist in Arabidopsis, including a family of 31 genes orthologous to the yeast copper chaperone ATX1, as well as homologues to the yeast copper chaperones CCS and COX17 [9].

Zinc

Adequate zinc concentration in plants ranges from 30 to 100 μg · g of dry weight⁻¹ [7]. Mechanisms of zinc uptake in plants are now emerging. Zinc is transported into roots by transporters of the ZIP family, which has more than 11 genes in Arabidopsis [8,10,11]. Our expression profile results (H. Wintz, T. Fox and C. Vulpe, unpublished work) show that some genes of the ZIP family are transcriptionally down-regulated by iron while others are down-regulated by zinc; however, the proteins themselves do not have a strict specificity for one single metal. Over-expression of one protein in response to one metal deficiency may lead to increased uptake of other metals. Such is the case for IRT1, an iron-regulated ZIP protein, that can also transport Zn and Mn [12] as well as other ZIP proteins [8]. Our expression profile results indicate that there is a strict and specific transcriptional regulation of the ZIP genes by either iron or zinc deficiencies. However, the profiles simultaneously support the idea that metal transport through ZIP family members has a leaky specificity. Our results also provide new data that some of the ZIP genes are up-regulated by both copper and zinc deficiencies. In addition to metal-specific regulation of ZIP gene expression, there is also tissue-specific regulation [6,8]. Our results show that some of the ZIP genes are expressed in roots but not in leaves. Intracellular transport and storage of both zinc and iron involve nicotianamine, which is synthesized by nicotianamine synthase. Nicotianamine synthase is coded for by a small family of genes consisting of four members that are also differentially regulated in a tissue-specific manner in response to zinc and iron.
Manganese transport and its regulation in bacteria
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Abstract
Regulation of manganese acquisition by bacteria occurs by both biochemical regulation of the activity of the transporters and transcriptional regulation of gene expression. Structural analysis suggests that calcium ions may regulate the function of an Mn ATP-binding cassette (ABC)-permease in *Synechocystis* 6803, a cyanobacterium, as well as in a number of other bacteria. The expression of genes encoding the manganese transporter in *Synechocystis* 6803 is regulated by a two-component signal-transduction mechanism that has not been previously observed for manganese and zinc transport in bacteria.

Introduction
Manganese is an essential trace element in almost all organisms. During oxygenic photosynthesis, Mn plays an essential role in the oxidation of water. We have previously identified and characterized an ATP-binding cassette (ABC)-permease complex which has a central role in the acquisition of Mn by the cyanobacterium *Synechocystis* 6803 [1,2]. This Mn ABC-permease consists of MntC, an extracellular solute-binding protein (SBP), MntB, a membrane-spanning protein, and MntA, an intracellular ABC protein. BLAST analysis with MntC as the probe identified a set of SBPs in a variety of bacteria. The corresponding transporters have been implicated in the transport of metal ions, primarily Mn and Zn [3,4].

Form of MntC
The protein sequences of five SBPs of ABC-permeases were compared using ClustalW alignment (Figure 1). MntC in *Synechocystis* 6803 and PsA in *Streptococcus pneumoniae* [5] are SBPs in Mn ABC-permeases, whereas TroA in *Treponema pallidum* [6] and ZnuA in *Escherichia coli* [7] are SBPs in Zn ABC-permeases. Figure 1 also includes an uncharacterized SBP in the Gram-negative bacterium *Haemophilus influenzae*. This protein is a close homologue of YfeA, the SBP component of an Fe- and Mn-uptake system in *Yersinia pestis* [8]. Residues corresponding to His-89, His-154 and Asp-295 in MntC are fully conserved in all five SBPs (Figure 1). All five of the