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REVIEW ARTICLE

ROLE OF ZINC AND MOLEBEDNUM: IN NUTRITION

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ABSTRACT

Zinc and molybdenum are involved in many biochemical processes supporting life. The most important of these processes are cellular respiration, cellular utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. zinc are involved in destruction of free radicals through cascading enzyme systems. Superoxide radicals are reduced to hydrogen peroxide by superoxide dismutases in the presence of zinc cofactors. On the other hand, excess intake of these trace elements leads to disease and toxicity; therefore, a fine balance is essential for health. Trace element--deficient patients usually present with common symptoms such as malaise, loss of appetite, anemia, infection, skin lesions, and low-grade neuropathy, thus complicating the diagnosis. Symptoms for intoxication by trace elements are general, for example, flu-like and CNS symptoms, fever, coughing, nausea, vomiting, diarrhea, anemia, and neuropathy. A combination of observation, medical and dietary history, and analyses for multiple trace elements is needed to pinpoint the trace element(s) involved. Serum, plasma, and erythrocytes may be used for the evaluation of zinc status, whereas only serum whole blood is preferred for molybdenum. The present review summarizes basic concepts of zinc and molebednum transport, use and storage and focuses on the zinc and molebednum system.

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INTRODUCTION

The human body has an elaborate system for managing and regulating the amount of key trace metals circulating in blood and stored in cells. Nutrient metals from our diet are incorporated into blood if blood levels are depleted, transported into cells if cellular levels are inadequate, or excreted if blood and cell levels are sufficient or overloaded. When this system fails to function properly, abnormal levels and ratios of trace metals can develop. One of the most common trace-metal imbalances is elevated copper and depressed zinc. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals (Kaslow, 2011). There are 2-4 grams of Zn distributed throughout the human body (Wapnir, 1990). Most zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye (Pfeiffer and Braverman, 1982). It is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes (Wapnir, 1990; Broadley *et al*., 2007). The body needs molybdenum (moh-LIB-den-um) for normal growth and health. For patients who are unable to get enough molybdenum in their regular diet or who have a need for more molybdenum, molybdenum supplements may be necessary (Barceloux, 1999). They are generally taken by mouth in multivitamin/mineral products but some patients may have to receive them by injection. Molybdenum is part of certain enzymes that are important for several body functions. A deficiency of molybdenum is rare. However, if the body does not get enough molybdenum, certain enzymes needed by the body are affected (Arslanoglu *et al*., 2001). This may lead to a build up of unwanted substances in some people.Molybdenum is an essential trace mineral in animal and human nutrition. It is found in several tissues of the human body and is required for the activity of some enzymes that are involved in catabolism, including the catabolism of purines and the sulfur amino acids. Molybdenum is a transition metal with atomic number 42 and an atomic weight of 95.94 daltons. Its symbol is Mo. Compounds of molybdenum are among the scarcer constituents of the earth's crust. In fact, molybdenum is only about three times more abundant than gold. The principal ore of molybdenum is molybdenite (molybdenum disulfide). Organic forms of molybdenum are found in living matter, from bacteria to animals, including humans (Chan *et al*., 1998; Welman A Shrader, Jr. 2004).

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Zinc

Zinc (Zn) is an essential micronutrient for plants as well as animals, playing catalytic or structural roles in enzymes,

transcription factors, ribosomes, and membranes. In humans, Zn deficiency is the second most common mineral nutritional disorder, affecting around 30% of the world's population. Deficiency include diarrhea, and wasting of body tissue. Eyesight, taste, smell and memory are also connected with zinc. The nutritional essentiality of zinc for the growth of living organisms had been recognized long before zinc biochemistry began with the discovery of zinc in carbonic anhydrase.

Properties of zinc

Zinc ion (Zn^{2+}) contains a filled d-orbital (d^{10}) and therefore does not participate in redox reactions but rather functions as a Lewis acid to accept a pair of electrons (Pfeiffer and Braverman, 1982). Therefore, the zinc ion is an ideal metal cofactor for reactions that require a redox-stable ion to function as a Lewis acid–type catalyst, such as proteolysis and the hydration of carbon dioxide. A very important property of Zn2+ that makes it well suited as a catalytic cofactor is that ligand exchange is rapid (Broadley *et al*., 2007), allowing for the rapid product dissociation required for efficient turnover. E.g. Carbonic anhydrase. In protein zinc-binding sites, the zinc ion is coordinated by different combinations of protein side chains, including the nitrogen of histidine, the oxygen of aspartate or glutamate and the sulfur of cysteine; among these, histidine is most commonly observed, followed by cysteine as sown in table given below.

Catalytic zinc	
Oxidoreductases[2]	
Alcohol dehydrogenase (horse liver)[3]	C_{37}
Alcohol dehydrogenase (*Thermoanaerobium brockii*)[4]	C_{37}
Hydrolases	
Carboxypeptidase A (bovine)[6,7]	H_{69}
Thermolysin (*Bacillus thermoproteolyticus*)[8,9]	H_{142}
DD carboxypeptidase (*Streptomyces albus*)[10]	H_{196}
Astacin (crayfish)[11]	H_{92}
β-Lactamase (*Bacillus cereus*)[12]	H_{86}
Cytidine deaminase (*Escherichia coli*)[13]	C_{132}
Alkaline phosphatase (*E. coli*)[14]	D_{327}
Adenosine deaminase (murine)[15]	H_{15}
Lyases	
Carbonic anhydrase II (human)[17,16]	H_{94}
Carbonic anhydrase (spinach)[18]	C_{213}
Carbonic anhydrase (*Methanosarcina thermophila*)[19]	H_{81}
Novel catalytic zinc sites	
Transferases	
Protein farnesyltransferase (rat)[20]	D_{297}
Cobalamin-dependent methionine synthase (*E. coli*)[21]	C_{247}
Cobalamin-independent methionine synthase (*E. coli*)[21,27]	H_{641}
Nonenzymatic	
Ada repair protein (*E. coli*)[22]	C_{38}
Structural zinc	
Alcohol dehydrogenase (horse liver)[3]	C_{97}
Aspartate carbamoyltransferase (*E. coli*)[23]	C_{109}
Zinc finger (Zif268) (mouse)[24]	C_7
Glucocorticoid receptor (rat)[25]	C_{440}
Ferrodoxin (*Sulfolobus* sp.)[26]	H_{16}

Zinc Transporters

With discoveries of proteins involved in cellular zinc homeostasis, it became clear how complex the control is. Dozens of transporters were identified and the specificity of most of these transporters is not well established. They are called zinc transporters, but several of them also transport other metal ions which are localized on the plasma membrane and on intracellular membranes. This extensive compartmentalization is characteristic for cellular zinc metabolism. The first mammalian zinc transporter gene, ZnT1, was identified by Palmiter and Findley, (1995). Before then, zinc transport in animals was viewed as occurring via co-transport as an anionic complex, as an amino acid (cysteine or histidine) chelate, or via the transferrin receptor route. Two protein families have now been implicated in zinc transport – Znt/SLC30 and Zip/ SLC39. SLC is group of membrane transport proteins include over 300 members and it is Solute-Linked Carrier where integer representing a family.

The SLC30/ZnT Family

The ZnT (solute-linked carrier 30 (SLC30)) proteins lower intracellular zinc by mediating zinc efflux from cells or influx into intracellular vesicles. The first ZnT protein (termed ZnT1) was isolated from a rat kidney cDNA library and shown to restore zinc resistance when transfected into a zinc sensitive baby kidney hamster (BHK) cell line under high levels of extracellular zinc. To date, the mammalian SLC30 family code for ten zinc transporters (ZnTs), ZnT1-10. Most members of this family have six predicted transmembrane domains (TMDs) and are predicted to have cytoplasmic amino and carboxy termini as shown in Figure 1. In addition, ZnTs characteristically harbour a long histidine-rich loop between TMD IV and V. ZnT proteins have been identified in intracellular compartments that are usually associated with endosomes, Golgi apparatus, or the endoplasmic reticulum. *Escherichia coli's* Yiip is a homolog of the human ZnT proteins residing in the inner membrane. Its 3-dimensional structure shows a dimeric protein with a transmembrane domain and a cytoplasmic domain and several zinc-binding sites in the monomer: a zinc site between the transmembrane helices involved in zinc transport, a zinc binding site between the two domains with unknown function, and a binuclear zinc binding site in the cytoplasmic domain which is thought to sense cytoplasmic zinc(II) ion concentrations and trigger a conformational change that allows transport of zinc through the membrane.

The SLC39/ZIP Family

The Zip (SLC39) proteins promote zinc transport from the extracellular fluid or from intracellular vesicles into the cytoplasm. ZIP transporters were first identified as "Zrt-, Irt-like Proteins" following their identification in yeast *Saccharomyces cerevisiae* (Zrt; zinc regulated transporter) and their similarity to the Fe (II) transporter Irt1 protein from the plant *Arabidopsis thaliana*. Since their discovery, the ZIP family of proteins has grown to more than 100 members including those from insects, bacteria, nematodes and mammals. Most ZIP transporters are predicted to have eight transmembrane (TMD) domains (Figure 1). Many of the family members have a long loop region between TMD III and IV that frequently contains a histidine-rich region that is suggested to be a putative zinc-binding domain. The majority of ZIP proteins share a similar predicted topology where both the N and C-termini are extracytoplasmic.

Role of Zinc

In zinc proteins, the major role of the zinc ion can be catalytic, cocatalytic or structural. In a catalytic zinc site, the zinc ion

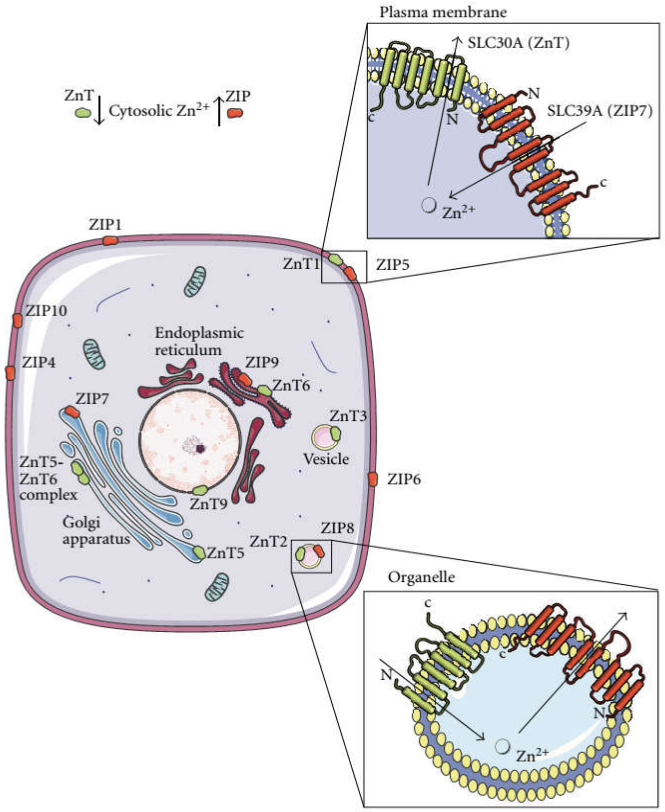


FIGURE 1: *Subcellular localization and direction of transport of the zinc transporter families, ZnT and ZIP.* Arrows show the direction of zinc mobilization for the ZnT (green) and ZIP (red) proteins. A net gain in cytosolic zinc is achieved by the transportation of zinc from the extracellular region and organelles such as the endoplasmic reticulum (ER) and Golgi apparatus by the ZIP transporters. Cytosolic zinc is mobilized into early secretory compartments such as the ER and Golgi apparatus by the ZnT transporters.

directly participates in the bond-making or –breaking step. In a cocatalytic zinc site, there are several metal ions bound in proximity to one another, where one plays a catalytic role and the other metal ions enhance the catalytic activity of the site (Keith A. McCall *et al*., 2000). Finally, in structural zinc sites, the zinc ion mainly stabilizes the tertiary structure of the enzyme in a manner analogous to disulfide bonds. In all cases, removal of the bound zinc can lead to a loss of enzymatic activity.

1.Catalytic zinc sites

A unique feature for a catalytic zinc site is the existence of an open coordination sphere. That is, the zinc-binding polyhedron contains at least one water molecule in addition to three or four protein ligands.

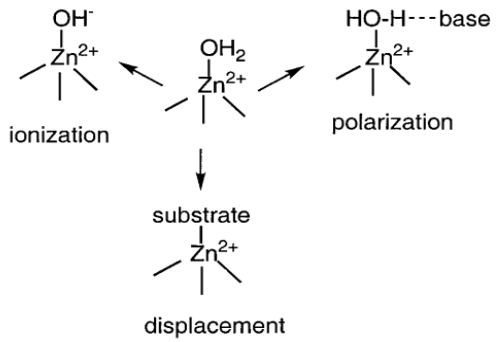


FIGURE 2

Figure 2. The zinc-bound water is a critical component for a catalytic zinc site, because it can be either ionized to zinc bound hydroxide (as in CA), polarized by a general base (as in carboxypeptidase A) to generate a nucleophile for catalysis or displaced by the substrate (as in alkaline phosphatase) (figure 2).

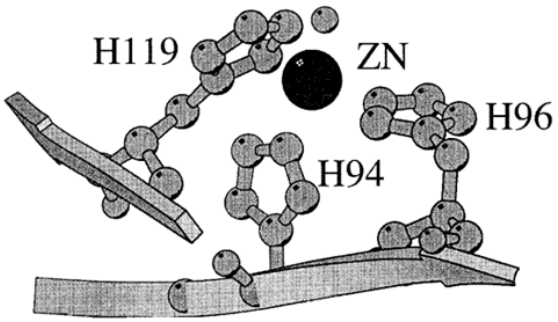


FIGURE 3

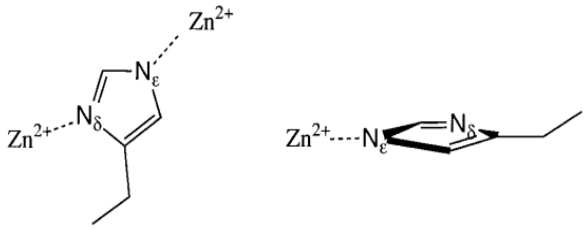


Figure 3. The majority of histidine zinc ligands found in zinc protein structures coordinate zinc through the N_ε atom, although coordination with N_δ atoms has also been observed as shown below in diagram. For these interactions, the metal ion prefers a head-on and in-plane approach to the nitrogen atom (Vedani and Huhta 1990).

Example of catalytic zinc site

Carbonic Anhydrase II: CA is a ubiquitous zinc metalloenzyme that catalyzes the reversible hydration of carbon dioxide. In mammals, more than seven isozymes have

been identified, and the isozyme CA II has the highest specific activity (Wapnir, 1990; Robert J. Cousins *et al*., 2006). CA II plays a variety of physiological roles, including promoting CO_2 exchange in the erythrocytes, kidney and lung; contributing to acid-base homeostasis; and promoting HCO_3^- secretion (Kaslow, 2011). The basic catalytic mechanism of CA was established from studies of bovine CA and human CAs I and II (Wapnir, 1990; Robert J. Cousins *et al*., 2006). Although additional CA isozymes and families have been discovered, the main features of the catalytic mechanism of the mammalian enzyme are retained (Stephen *et al*., 2012).

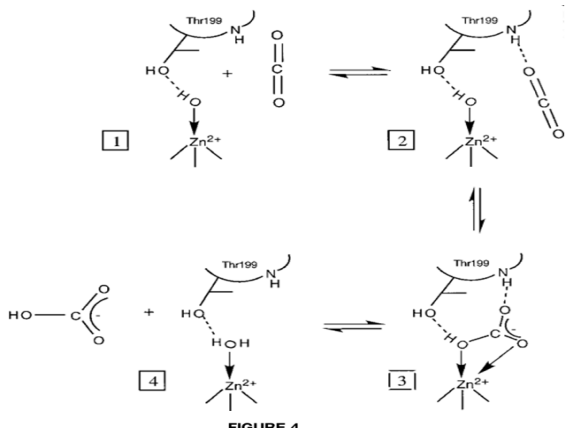


Figure 4. The mechanism of CO_2 hydration, catalyzed by CA II, can be separated into two steps . In the first step, zinc bound hydroxide attacks the carbonyl carbon of CO_2 to form zinc-bound bicarbonate; bicarbonate is subsequently displaced with water by a ligand-exchange step. In the second step, H^+ is transferred from zinc-bound water to external buffer via a shuttle group (H_{64} in CA II) to regenerate the catalytically active species, the zinc-bound hydroxide

Carboxypeptidase A. It is a metalloexopeptidase containing one zinc ion bound to a single polypeptide chain of 307 amino acids. Its function is the hydrolysis of C-terminal amino acids from polypeptide substrates, and it exhibits a preference toward those substrates possessing large, hydrophobic C-terminal side chains such as phenyl- alanine. Important residues for catalysis and binding are Glu-270, Arg-71, Arg-127, Asn-144, Arg-145, Tyr-248, Zn2+, and the zinc-bound water molecule (Figure 5).

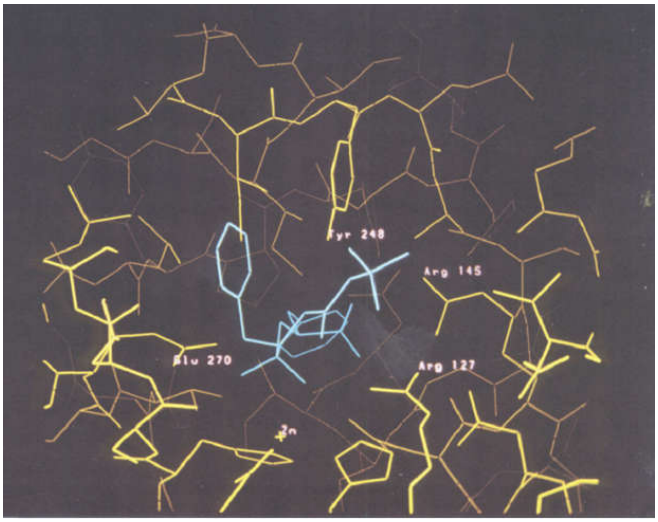


Figure 5. Important residues for catalysis and binding are Glu-270, Arg-71, Arg-127, Asn-144, Arg-145, Tyr-248, Zn2+, and the zinc-bound water molecule

Cocatalytic zinc sites

In multimetal enzymes, the two or more zinc (or other metal) atoms may operate in concert to enhance catalysis. A class of catalytic zinc sites, called cocatalytic zinc sites, has been defined in which two or more zinc atoms are in close proximity to one another. This group of enzymes includes alkaline phosphatase (with two zinc ions and one magnesium ion), phospholipase C (three zinc ions), nuclease P1 (three zinc ions) and leucine aminopeptidase (two zinc ions). A representative structure (phospholipase C) is shown in Figure 6. The first zinc ion, designated as the catalytic zinc (Zn1in Fig. 6), contains a bound water that is essential for catalysis and has an His_2Glu metal polyhedron similar to those found in other single catalytic zinc sites. The second zinc (Zn2) and the third metal (Zn3/Mg) ion sites may have unusual ligands such as the oxygen of serine or threonine or the nitrogen of the N terminal amino group. An additional distinctive feature is the existence of one or more bridging ligands (either aspartate or water or both) between the second zinc ion (Zn2) and the third zinc ion (Zn3) or magnesium ion (Zn3/Mg). These sites are termed "cocatalytic" because all three metals play crucial roles in catalysis despite only the zinc activating the attacking water being termed "catalytic."

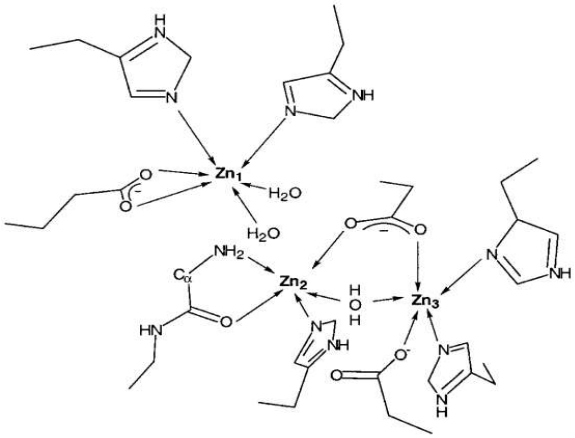


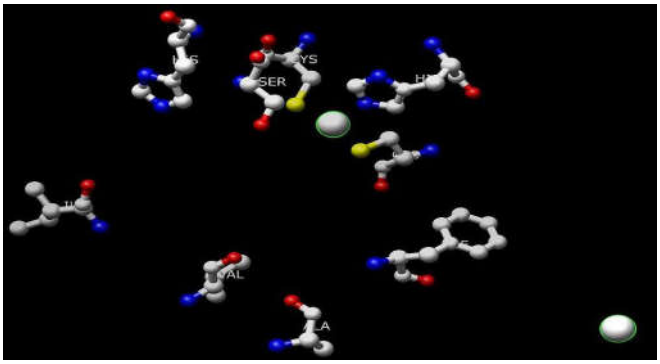
FIGURE 6 Example of cocatalytic zinc site: phospholipase C (Hough et al. 1989). In phospholipase C, as in nuclease P1, the back-bone amino and carbonyl groups of N-terminal Trp, coordinate Zn2.

Structural zinc sites

In structural zinc sites, the metal ion is coordinated by four amino acid side chains so that solvent is excluded as an inner sphere ligand. Cysteine is by far the ligand observed most frequently in these sites, with histidine also being present in many cases and aspartate being present in one case. Alcohol dehydrogenase, Zinc finger (Zif268) are the example of structural zinc sites.

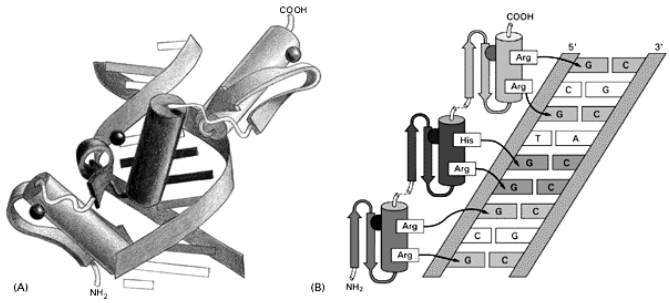
Example of Structural zinc sites

Alcohol Dehydrogenase: Alcohol dehydrogenases (ADH) are a group of dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of NAD+ to NADH. In humans and many other animals, they serve to break down alcohols that otherwise are toxic, and they also participate in generation of useful aldehyde, ketone, or alcohol groups during biosynthesis of various metabolites. In yeast, plants, and many bacteria, some alcohol dehydrogenases catalyze the opposite reaction as part of fermentation to ensure a constant supply of NAD+.



The active site of human ADH1 consists of a zinc atom, His-67, Cys-174, Cys-46, Thr-48, His-51, Ile-269, Val-292, Ala-317, and Leu-319. The zinc is coordinated by Cys-46, Cys-174, and His-67 (as shown below).

Zinc Finger. Miller *et al*., (1985) observed that *Xenopus laevis* transcription factor IIIA contains 9 repetitive sequences of C (cysteine) and H (histidine) residues that are involved in the binding of 9 zinc ions. This characteristic ligand signature of metal-coordinating amino acid side chains interspersed with non-coordinating amino acids forms relatively small protein domains that interact with nucleic acids like the fingers of a hand gripping a rod and hence became known generically as a "zinc finger." Zinc finger proteins are among the most abundant proteins in eukaryotic genomes. Structures have recently been reported for many new zinc finger domains with novel topologies, providing important insights into structure/function relationships. In addition, new structural studies of proteins containing the classical Cys(2)His(2) zinc finger motif have led to novel insights into mechanisms of DNA binding and to a better understanding of their broader functions in transcriptional regulation.



Zinc finger structures are as diverse as their functions. The most common "fold groups" of zinc fingers are the Cys2His2-like (the "classic zinc finger"), treble clef, and zinc ribbon etc. When the entire human genome was scanned for zinc binding signatures, it was predicted that at least 3-10% of the genes encode zinc proteins. The number of zinc proteins correlates linearly with the number of genes in a particular genome and is higher in eukarya (8.8%) than in bacteria and archaea (5–6%). The increase in the zinc proteome in eukaryote is due to the large number of additional zinc proteins involved in regulation. Such as the zinc-binding domains of zinc finger proteins that extend considerably the conformational landscape of proteins and hence the possibilities for interactions.

Zinc in regulation: zinc (II) ions as signaling ions

Perhaps the most exciting area in contemporary zinc biology is the role of released zinc (II) ions in information transfer within cells and between cells. The preference of zinc (II) ions for specific coordination environments in proteins endows these signals with a certain degree of specificity. The amplitudes of zinc (II) ion signals determine which proteins are targeted because any target must have a commensurate affinity for zinc. Under conditions of stimulation, cellular zinc (II) ion concentrations increase and reach concentrations of >2 nmol/L only under oxidative stress and in apoptosis. In addition to the release of zinc from proteins, two pathways of vesicular release of zinc (II) ions increase the concentrations of free zinc (II) ions. The first one is the release of zinc from vesicles into the extracellular space. The prime example is the release of zinc (II) ions from synaptic vesicles in a subset of glutamatergic neurons (Figure 7). One target of the released zinc (II) ions is the postsynaptic N-methyl-D-aspartate receptor, which zinc inhibits at low nano moles per liter concentrations. Zinc (II) ions also modulate the activities of other neuroreceptors, and there is reuptake with functional effects in the presynaptic cell which targets of zinc (II) ions are physiologically important depends on the effective concentrations of synaptic zinc.

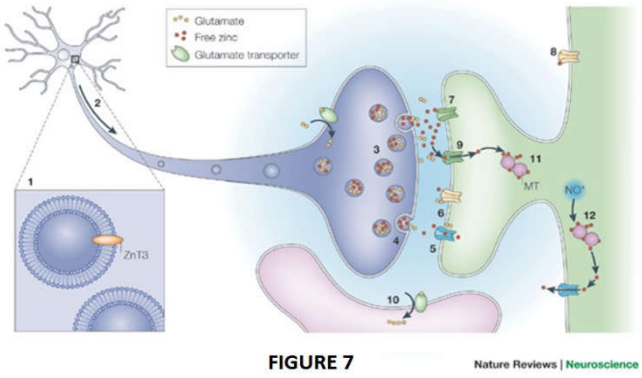


Figure 7. In addition to the release of zinc from proteins, two pathways of vesicular release of zinc (II) ions increase the concentrations of free zinc (II) ions. The first one is the release of zinc from vesicles into the extracellular space. The prime example is the release of zinc (II) ions from synaptic vesicles in a subset of glutamatergic neurons.

Zinc (II) ions also occur in other nonsynaptic vesicles and are released from cells by exocytosis from these vesicles that are loaded with zinc (II) ions by different zinc transporters, ZnT3 in neurons in the brain, ZnT8 in pancreatic β cells, or ZnT2 in epithelial cells of the mammary gland. The second pathway involves intracellular release of zinc (II) ions from vesicles. Stimuli targeting the zinc transporter Zip7 control the release of zinc (II) ions from a store in the ER to the cytoplasm. The pathway involves growth factor stimulation and casein kinase 2–induced phosphorylation of Zip7, which opens the store for zinc release. In macrophages, stimulation of the immunoglobulin E receptor and extracellular signal-regulated kinase/inositol triphosphate signaling triggers zinc release, which is preceded by Ca^{2+} release. This phenomenon has been referred to as a "zinc wave. Two modes of zinc signaling: "early zinc signaling" (EZS) and "late zinc signaling" (LZS). EZS involves transcription-independent mechanisms where an extracellular stimulus directly induces an increase in zinc levels within several minutes by releasing zinc from intracellular stores (e.g., endoplasmic reticulum). The phenomenon of Zinc Wave comes under the early zinc signaling. LSZ is induced several hours after an external stimulus and is dependent on transcriptional changes in zinc transporter expression (Wolfgang Maret, 2013) (Figure 8).

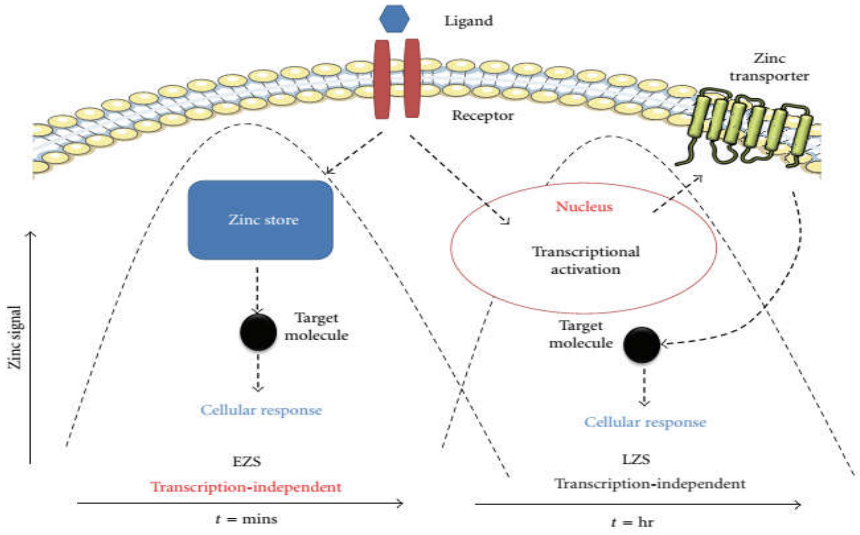


Figure 8. Two modes of zinc signaling: "early zinc signaling" (EZS) and "late zinc signaling" (LZS). EZS involves transcription-independent mechanisms where an extracellular stimulus directly induces an increase in zinc levels within several minutes by releasing zinc from intracellular stores (e.g., endoplasmic reticulum). The phenomenon of Zinc Wave comes under the early zinc signaling. LSZ is induced several hours after an external stimulus and is dependent on transcriptional changes in zinc transporter expression (15).

Zinc transporter	Cellular localization	Tissue expression	Disease associations
SLC39A1/ZIP1	Plasma membrane	Ubiquitously expressed	Prostate cancer
SLC39A2/ZIP2	Plasma membrane	Blood, prostate	Carotid artery disease
SLC39A3/ZIP3	Plasma membrane, intracellular compartments	Mammary gland, prostate	Unknown
SLC39A4/ZIP4	Apical membranes	Small intestine, stomach, colon, kidney, brain	Pancreatic cancer, acrodermatitis enteropathica (AE)
SLC39A5/ZIP5	Basolateral membranes	Pancreas, kidney, liver, spleen, colon, stomach	Unknown
SLC39A6/ZIP6	Plasma membrane	Ubiquitously expressed	Breast cancer
SLC39A7/ZIP7	Golgi apparatus, endoplasmic reticulum	Ubiquitously expressed	Breast cancer
SLC39A8/ZIP8	Vesicles	Ubiquitously expressed	Unknown
SLC39A9/ZIP9	Trans-Golgi network	Ubiquitously expressed	Unknown
SLC39A10/ZIP10	Plasma membrane	Ubiquitously expressed	Breast cancer
SLC39A11/ZIP11	Unknown	Mammary gland	Unknown
SLC39A12/ZIP12	Unknown	Retina, brain, testis, lung	Schizophrenia
SLC39A13/ZIP13	Golgi apparatus	Ubiquitously expressed	Ehlers-Danlos syndrome
SLC39A14/Zip14	Plasma membrane	Ubiquitously expressed	Asthma
SLC30A1/ZnT1	Plasma membrane	Ubiquitously expressed	Alzheimer's disease, Pancreatic cancer
SLC30A2/ZnT2	Vesicles, lysosomes	Pancreas, kidney, testis, epithelial cells, small intestine	Unknown
SLC30A3/ZnT3	Synaptic vesicles	Brain, testis	Alzheimer's disease
SLC30A4/ZnT4	Intracellular compartments	Mammary gland, brain, small intestine, placenta, blood, epithelial cells	Alzheimer's disease
SLC30A5/ZnT5	Secretory vesicles, Golgi apparatus	Ubiquitously expressed	Osteopenia
SLC30A6/ZnT6	Secretory vesicles, Golgi apparatus	Small intestine, liver, brain, adipose tissue	Alzheimer's disease
SLC30A7/ZnT7	Golgi apparatus	Retina, small intestine, liver, blood, epithelial cells, spleen	Prostate cancer
SLC30A8/ZnT8	Secretory vesicles	Pancreatic B-cells	Type 1 and 2 diabetes mellitus
SLC30A9/ZnT9	Cytoplasm, nucleus	Ubiquitously expressed	Unknown
SLC30A10/ZnT10	Unknown	Liver, brain	Parkinson's disease, dystonia, liver disease

Zinc deficiency

As Zinc is cofactor to over 300 enzymes and over 1000 transcription factors so its deficiency can cause the alteration of function of these enzymes and cofactors. Zinc deficiency can cause retardation, cessation of growth, impaired wound healing, hair loss or defects leading to reproductive failure.

Zinc toxicity

Even though zinc is an essential requirement for a plants and animals, excess zinc can be harmful, and cause zinc toxicity. In humans such toxicity levels have been seen to occur at ingestion of greater than 225 mg of Zinc. The free zinc ion in solution is highly toxic to bacteria, plants, invertebrates, and even vertebrate fish. Nausea, vomiting, pain, cramps and diarrhea are some symptoms of zinc toxicity. There is also a condition called the "zinc shakes" or "zinc chills" or metal fume fever that can be induced by the inhalation of freshly formed zinc oxide formed during the welding of galvanized materials. The free zinc ion is also a powerful Lewis acid up to the point of being corrosive. When ingested, metallic zinc is readily dissolved by the hydrochloric acid of the stomach, and thus gives the corrosive zinc chloride. For example, swallowing a post-1982 American one cent piece (97.5% zinc) can cause damage to the stomach lining due to the high solubility of the zinc ion in the acidic stomach.

Molybdenum

Molybdenum is a transition metal, which exists predominantly in the form of the oxyanion molybdate, which serves as an essential micronutrient in all kingdoms of life. Yet, molybdate alone does not exhibit biological activity, but is bound to an organic pterin backbone, which upon binding of molybdate is converted into the molybdenum cofactor (Moco). In the form of molybdate the transition metal molybdenum is essential for plants as it is required by a number of enzymes that catalyze key reactions in nitrogen assimilation, purine degradation, phytohormone synthesis, and sulfite detoxification. With the exception of the nitrogenase cofactor, molybdenum is incorporated into proteins as the molybdenum cofactor that contains a mononuclear molybdenum atom coordinated to the sulfur atoms of a pterin derivative named molybdopterin.

Molybdenum enzymes generally catalyze the transfer of an oxygen atom (ultimately derived from or incorporated into water) to or from a substrate. Molybdenum has a versatile redox chemistry that is used by the enzymes to catalyze diverse redox reactions. Each reaction, either reduction or oxidation, involves the transfer of two electrons, thereby causing a change in the oxidation state of the molybdenum atom in the substrate binding site from IV to VI or vice versa. In soil, a critical point concerns the bioavailability of molybdate, which is favored above pH 5.5 and impaired at lower pH due to the adsorption of molybdate to soil oxides (Barceloux, 1999). Under low-pH conditions, molybdate assimilation is therefore limited resulting in molybdenum deficiency associated with reduced molybdo-enzyme activities and reductions in plant growth and yield. In contrast, molybdenum toxicity by oversupply of plants with molybdate is extremely rare and characterized by relatively mild symptoms such as yellowish leaves (Barceloux, 1999) or reduced seedling growth and increased anthocyanin concentrations (Arslanoglu *et al*., 2001).

Uptake and transport of molybdate

The active uptake of exogenously applied molybdate by roots and its transport to the shoot. However, molybdate levels reached a maximum in the shoot 6 h after application which indicating that uptake of molybdate and sensing of intracellular molybdate levels are well controlled processes. Notably, when molybdate is applied solely to leaves transport also occurs downward to the stem and roots, demonstrating that molybdate is a highly mobile compound translocated between various plant tissues. Furthermore, the finding that sulfate is a potent inhibitor of molybdate uptake (Florian Bittner, 2014; Barceloux, 1999) that in turn low sulfate concentrations in the soil promote molybdate uptake (Caroline Kisker *et al*., 1997). In fact sulfate and molybdate share a high degree of similarity as they both possess a double negative charge (SO42−, MoO42−), are similar in size, and have tetrahedral structures. It was proposed that molybdate import and distribution are facilitated by sulfate transporters or related systems. The first identified molybdate-specific transporters from *Chlamydomonas reinhardtii* (Ralf R. Mendel, 2013) and *Arabidopsis thaliana* (Caroline Kisker *et al*., 1997; Florian Bittner, 2014) indeed turned out to belong to the family of sulfate transporters, referred to as MOT1 (Figure 9).

In addition to MOT1, another molydate transporter of the sulfate transporter family, MOT2, has been identified in *Arabidopsis* (Ralf R. Mendel, 2013). MOT2 localizes to the vacuole and MOT2-deficient plants are characterized by accumulation of molybdenum in leaves and reduced molybdenum contents in seeds.

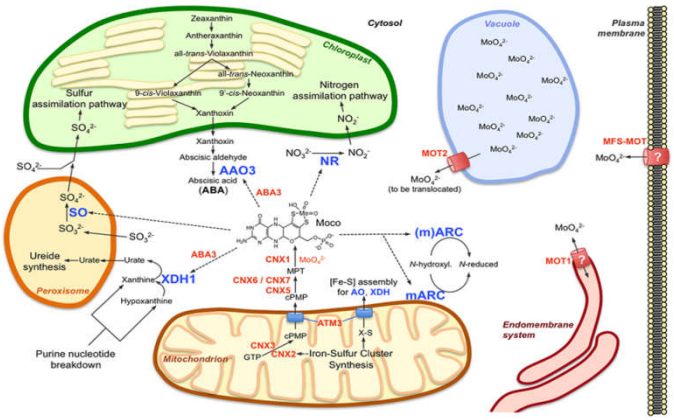


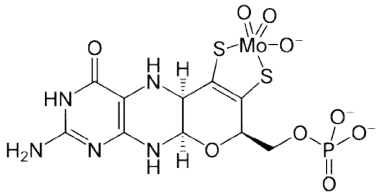
Figure 9. Uptake and transport of molybedate in plant cell

The plant homolog (MFS-MOT) of the major facilitator superfamily molybdate transporter MOT2 from *Chlamydomonas* might be required for molybdate import across the plasma membrane. A novel molybdate transporter, also denoted as MOT2, has been identified in *Chlamydomonas* (Barceloux, 1999; Chan *et al*., 1998). In contrast to Arabidopsis MOT2 however, this transporter is a member of the major facilitator superfamily which is completely unrelated to the sulfate transporter family.

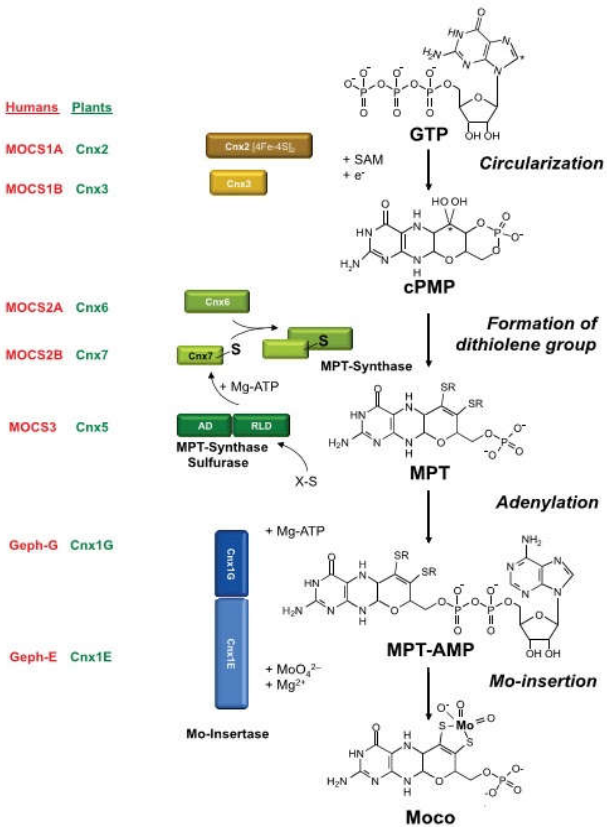
The Molybdenum Cofactor

Molybdenum is not directly attached to the catalytic site; rather, the atom is complexed within a specific low molecular scaffold to fulfill its catalytic function.

This compound is a unique tricyclic pterin called molybdopterin or metal-containing pterin (MPT).

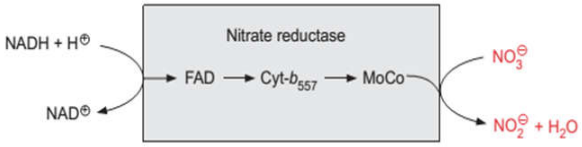


As the result of molybdenum coordination by MPT, Moco is formed. Moco shares its cofactor with a family plant enzymes that are involved in sulfite detoxification, purine catabolism, and abscisic acid biosynthesis. Task of the pterin moiety of Moco - Pterin may have evolved to position the catalytic metal correctly within the active center of a given molybdenum enzyme. Another possible role of the pterin moiety could be control of the redox behavior of the molybdenum atom. In addition, the pterin might also participate in the electron transfer to or from molybdenum via the delocalized electrons within the pterin. X-ray crystallographic analyses of molybdenum enzymes revealed that the cofactor is not located on the surface of the protein but is buried deeply within the interior of the enzyme, and a tunnel-like structure makes it accessible to the cognate substrates (Ralf R. Mendel, 2013). In all higher organisms studied so far, Moco is synthesized by a conserved biosynthesis pathway that can be divided into four steps according to the biosynthetic intermediates cyclic pyranopterin monophosphate (cPMP), MPT, adenylated MPT (MPT-AMP), and Moco, respectively. In eukaryotes, six proteins catalyzing Moco biosynthesis have been identified in plants, fungi, and humans.Different nomenclatures were introduced for genes and gene products involved in Moco formation. Genes and the encoded proteins were named in plants according to the cnx nomenclature (cofactor for nitrate reductase and xanthine dehydrogenase). For human Moco synthetic genes, a different MOCS (molybdenum cofactor synthesis) nomenclature was introduced.

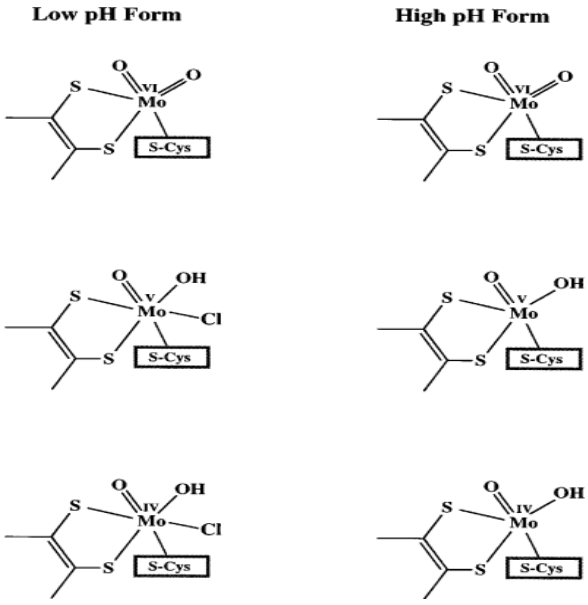


Molybdenum-dependent enzymes

Among the molybdo-enzymes, nitrate reductase (NR) represents the cytosolic key enzyme of nitrogen assimilation that reduces nitrate to nitrite (Ralf R. Mendel, 2013). In addition to Moco, NR also depends on heme and FAD as prosthetic groups and strictly requires NADH or NADPH for enzymatic activity. A deficiency in NR results in the inability of the plant to mobilize nitrogen, which is inseparably associated with the loss of plant viability in the absence of alternative nitrogen sources. Under low-oxygen conditions, NR is capable of reducing nitrite to nitric oxide (NO), and NR-derived NO appears to be among the major sources of NO in plants (Caroline Kisker *et al*., 1997; Florian Bittner, 2014). Nitrate reductase of higher plants consists of two identical subunits. Each subunit contains an electron transport chain consisting of one (FAD), one cyt-b557, and one cofactor containing molybdenum.



Plant sulfite oxidase (SO): is a peroxisomal enzyme (Welman A Shrader, Jr. 2004), which exclusively consists of a Moco-binding domain required for oxidizing sulfite to sulfate. In this process, substrate derived electrons are transferred to molecular oxygen with formation of superoxide anions (Caroline Kisker *et al*., 1997) and hydrogen peroxides (Ralf R. Mendel, 2013). SO represents a part of an intracellular sulfite homeostasis network required to prevent plant cells from the toxic effects of endogenously arising sulfite (Barceloux, 1999; Arslanoglu *et al*., 2001). The coordination environment of the Mo seems to be very sensitive to the oxidation state of the molybdenum and to the pH of the solution. At pH 9.0, all three oxidation states of the molybdenum possess five ligands which is suitable for plant sulfite oxidase. At pH 6.0, an additional ligand, most probably chloride, is observed in the Mo (V) and Mo (IV) states. When the enzyme is reduced from the Mo (VI) to the Mo (IV) state, one of the oxygen ligands will be protonated.

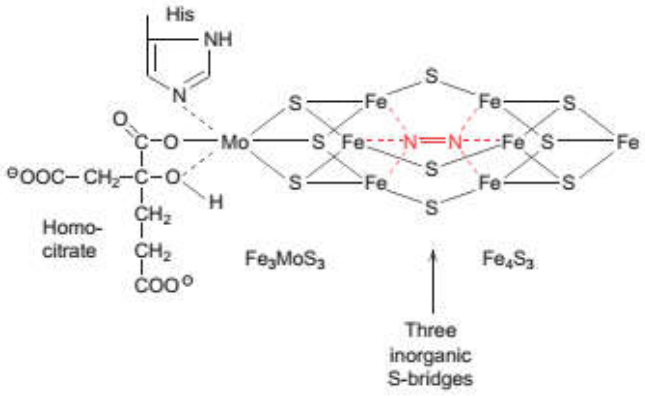


Xanthine dehydrogenase (XDH): requires Moco, FAD, and two iron-sulfur clusters (Caroline Kisker *et al*., 1997), and its main function is associated to purine degradation by oxidizing hypoxanthine to xanthine and xanthine to uric acid in the cytosol. Electrons released from the substrate are preferably transferred to NAD+. At extremely low concentrations of NAD^+, molecular oxygen can serve as alternative electron acceptor with simultaneous generation of superoxides (Arslanoglu *et al*., 2001). As indicated by XDH-deficient plants, the function of XDH is crucial for plant growth, senescence, and fertility (Ralf R. Mendel, 2013). Independent from other substrates, XDH exhibits strong intrinsic NADH oxidase activity, which is accompanied by the use of oxygen as electron acceptor and simultaneous formation of superoxide anions (Florian Bittner, 2014). It is speculated that this activity is of importance in the response to biotic and abiotic stresses.

Aldehyde oxidase (AO): has derived from XDH (Chan *et al*., 1998) and therefore shares catalytical and structural similarities with XDH. In contrast to XDH however, AO proteins preferably oxidize aldehydes to the respective carboxylic acid. The genome of most, if not all, plant species harbors several AO genes, which indicates a specific need of plants for several independent aldehyde oxidation activities. The number of AO proteins and their specific functions might therefore relate to the specific metabolic and environmental demands of different plant species. For instance, the Arabidopsis genome encodes the genes AAO1–AAO4 and the respective proteins form homo- and hetero-dimers with overlapping, but also distinct substrate specificities. The most important isoform is AAO3, which catalyzes the oxidation of abscisic aldehyde to abscisic acid (ABA) in the last cytosolic step of ABA synthesis. Due to the function of ABA in many aspects of plant growth and development, and in adaptation to a variety of abiotic stresses, AAO3-deficient plants with reduced ABA levels are characterized by a high transpiration rate, reduced stress tolerance, and impaired seed dormancy (Welman A Shrader, Jr. 2004). For AAO1 and AAO2, a function in one of the multiple biosynthesis routes of indole-3-acetic acid is suggested as they both efficiently catalyze the oxidation of indole acetaldehyde to indole-3-acetic acid in vitro. AAO4 is expressed preferably in siliques and catalyzes the oxidation of benzaldehyde into benzoic acid, the latter being incorporated into glucosinolates that likely serve as herbivore defense compounds (Florian Bittner, 2014).

The mitochondrial amidoxime reducing component (mARC): has been identified in mitochondria of mammals and catalyzes the reduction of N-hydroxylated substances (Ralf R. Mendel, 2013). Like mammals, plant genomes encode two mARC isoforms, which have not yet been investigated in detail. The physiological role of mARC proteins is therefore still enigmatic, even though previous studies in *Chlamydomonas* and on recombinant human proteins suggest a function in the detoxification of N-hydroxylated base analogs (Ralf R. Mendel, 2013) and/or in the regulation of L-arginine-dependent NO synthesis. Although the physiological functions of some molybdo-enzymes are as yet not fully understood, it is obvious that others hold key positions in essential or at least important metabolic pathways. Any factor that affects one of these enzymes will thus also affect the respective pathway, with effects on Moco biosynthesis and molybdate supply resulting in the pleiotropic impairment of all molybdo-enzyme activities, associated with severe reduction of plant viability or even death of the plant.

Nitrogenase Complex: catalyzes the reduction of nitrogen (N2) under ambient conditions. The molybdenum-dependent nitrogenase consists of a reductase component (NifH) which contain two binding sites for ATP and a catalytic component (NifDK). The active site of molybdenum nitrogenase is called the iron-molybdenum (FeMo) cofactor or the M-cluster. Located within NifDK (a α2β2-tetramer), the M-cluster receives electrons from NifH (a ϒ2-dimer) in an ATP-assisted process and subsequently serves as the site for substrate reduction upon accumulation of sufficient electrons. M-cluster can be viewed as (Fe4S3) and (MoFe3S3) sub clusters bridged by three sulfide atoms.



Additionally, it has a homocitrate moiety attached to its molybdenum end, as well as a carbide atom coordinated in the center of its structure. The M-cluster is ligated to the α-subunit of NifDK by $Cys^{\alpha 275}$ at its iron end and Hisα442 at its molybdenum end, with Lysα426 providing an additional anchor for its homocitrate entity. When reductase component reduced, two molecules of ATP bind to it, resulting in a conformational change of the protein, by which the redox potential of the 4Fe-4S cluster is comes from -0.25 to -0.40 V. Following the transfer of an electron to nitrogenase, the two ATP molecules bound to the protein are hydrolyzed to ADP and phosphate, and then released from the protein. Thus with the consumption of two molecules of ATP, one electron is transferred from NADH to nitrogenase by nitrogenase reductase.

Interrelation between Molybdenum and Iron

The molybdate supply significantly increases the capacity of tomato plants to absorb Fe^{2+}. The inverse phenomenon, an influence of Fe^{2+} on molybdate uptake, has later been investigated in excised rice roots, which showed increased molybdate uptake capacity in the presence of $FeSO_4$. This interrelation was confirmed in an ionomics study involving iron deficient plants, in which molybdenum contents were shown to be reduced (Yilin Hu and Markus W. Ribbe, 2013). Apart from metal uptake, crosstalk between molybdenum and iron is observed on the levels of Moco biosynthesis and molybdo-enzymes. Moco biosynthesis enzyme CNX2 requires two (4Fe-4S) clusters for activity and the crosstalk between molybdenum and iron is further substantiated by the fact that CNX2 gene expression is controlled by both, molybdate and iron availability. As sulfur is embedded into molybdenum metabolism also at several other steps (e.g., Moco synthesis, sulfite detoxification, and molybdate uptake), the three nutrients molybdenum, iron and sulfur appear to interact closely on various levels within a common metabolic network that needs to be elucidated in future studies.

Many players in the molybdenum homeostasis network have meanwhile been identified and characterized with the enzymes of Moco synthesis and the molybdo-enzymes. Understanding of the function of molybdate transporters has just begun and a lot more effort needs to be invested to depict the import and export routes in plants and to identify the candidate(s) responsible for uptake of molybdate at the root: soil interface. Moreover, very little is known about factors controlling the expression of genes and interaction of proteins involved in molybdenum homeostasis, thus requiring further in-depth analysis on the transcriptome, proteome, metabolome, and ionome level.

Molybdenum deficiency

It is a rare human disease in which the absence of molybdenum cofactor leads to accumulation of toxic levels of sulphite and neurological damage. Usually this leads to death within months of birth, due to the lack of active sulfite oxidase. Furthermore, a mutational block in molybdenum cofactor biosynthesis causes absence of enzyme activity of xanthine dehydrogenase/oxidase and aldehyde oxidase. When caused by a mutation in the MOCS1 gene it is the type a variant. It can also be caused by a mutation in the MOCS2 gene or the GEPH gene.

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