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REVIEW

Cell biology of molybdenum in plants

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Abstract The transition element molybdenum (Mo) is of essential importance for (nearly) all biological systems as it is required by enzymes catalyzing important reactions within the cell. The metal itself is biologically inactive unless it is complexed by a special cofactor. With the exception of bacterial nitrogenase, where Mo is a constituent of the FeMo-cofactor, Mo is bound to a pterin, thus forming the molybdenum cofactor (Moco) which is the active compound at the catalytic site of all other Mo-enzymes. In plants, the most prominent Mo-enzymes are nitrate reductase, sulfite oxidase, xanthine dehydrogenase, aldehyde oxidase, and the mitochondrial amidoxime reductase. The biosynthesis of Moco involves the complex interaction of six proteins and is a process of four steps, which also includes iron as well as copper in an indispensable way. After its synthesis, Moco is distributed to the apoproteins of Mo-enzymes by Moco-carrier/binding proteins that also participate in Moco-insertion into the cognate apoproteins. Xanthine dehydrogenase and aldehyde oxidase, but not the other Mo-enzymes, require a final step of posttranslational activation of their catalytic Mo-center for becoming active.

Keywords Molybdenum cofactor · Sulfite oxidase · Nitrate reductase · Xanthine dehydrogenase · Aldehyde oxidase · Molybdenum cofactor deficiency

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Introduction

The transition element molybdenum (Mo) occurs in a wide range of metalloenzymes in bacteria, fungi, algae, plants and animals where it forms part of the active centers of these enzymes (for reviews see Schwarz and Mendel 2006; Bittner and Mendel 2010). In order to gain biological activity, Mo has to be complexed by a pterin compound thereby forming the prosthetic group named molybdenum cofactor (Moco) (for reviews see Schwarz et al. 2009; Mendel and Schwarz 2011). It has been long known that Mo (Bortels 1930) is an essential nutrient for plants, animals and microorganisms. Mo is very abundant in the oceans in the form of the MoO_4^{2-} anion. Furthermore, in soils too, the molybdate anion is the only form of Mo that is available for plants and bacteria. Biologically, Mo belongs to the group of trace elements, i.e. the organism needs it only in minute amounts. If, however, an organism takes up too high amounts of Mo, toxicity symptoms are observed (Turnlund 2002). On the other side, unavailability of Mo, is lethal for the organism. More than 50 Mo-containing enzymes are known, most of them of bacterial origin, while only a handful Mo-enzymes were found among eukaryotes. In plants, five Mo-enzymes are known to this end: nitrate reductase (NR), sulfite oxidase (SO), xanthine dehydrogenase (XDH), aldehyde oxidase (AO) and mitochondrial amidoxime reductase (mARC). In addition to the pterin-type of cofactor, there is another type of Mo-containing cofactor, which is found only once in nature, namely in bacterial nitrogenase, forming the so-called iron-molybdenum cofactor, FeMoco. Nitrogenase is required for biological nitrogen fixation, which is an essential step in the nitrogen cycle in the biosphere. In contrast to nitrogenase, all other Mo-containing enzymes characterized to this end contain the pterin-type cofactor (Table 1).

Table 1 Mo-enzymes in plants (*Arabidopsis thaliana*)

Mo-enzyme	Number of genes	Subcellular location	Function	ROS/RNS side product
Nitrate reductase	2	Cytosol	Nitrate assimilation	NO
Sulfite oxidase	1	Peroxisome	Sulfite detoxification	H_2O_2
Xanthine dehydrogenase	2	Cytosol (peroxisome)	Purine degradation, NADH oxidase	Superoxide anions
Aldehyde oxidase	4	Cytosol	Synthesis of ABA (auxins)	H_2O_2, superoxide anions
Mitochondrial amidoxime reductase	2	Mitochondria	Detoxification (?)	n.d.

This article will first present those plant enzymes that need Mo as catalytic metal, followed by a more cell biological part that will describe the way that Mo takes from uptake into the cell, via formation of the Moco and its storage, to its final insertion into apo-metalloenzymes. Most of our knowledge about eukaryotic Mo metabolism derives from studies in plants. This may be surprising, but in pre-genomic times the detailed characterization of NR-deficient mutants (using callus cultures and in vitro-plants) contributed substantially to our understanding of the genetics and biochemistry of Moco biosynthesis in plants (summarized by Müller and Mendel 1989). These NR-deficient mutants fell into two groups: those with a defect in the NR-apoprotein and others which were defective in Moco. The identification of several genetic complementation groups among Moco-deficient plant mutants (using crossing methods but also protoplast fusions) and the conserved structure of Moco provided a basis to propose an evolutionary old multi-step pathway for the biosynthesis of Moco as prosthetic group needed by all Mo-enzymes (Mendel 1992). The molecular, biochemical and genetic analysis of Moco mutants in higher plants formed the basis to decipher Moco biosynthesis also in humans where Moco deficiency is a severe genetic disease with fatal consequences for the affected individuals (Reiss and Johnson 2003). All higher plants possess Mo-enzymes, however, genome-wide database analyses revealed a significant number of bacteria and unicellular lower eukaryotes that do not need Mo. Loss of Mo-utilization is obviously connected to a host-associated life-style that makes Mo-enzymes unnecessary, while all multi-cellular eukaryotes are dependent on Mo (Zhang and Gladyshev 2008). Therefore model yeasts like *Saccharomyces cereviseae* and *Schizosaccharomyces pombe* play no role in Mo research as they belong to those organisms that do not contain Mo-enzymes while other yeasts such as *Pichia pastoris* do need Moco.

What enzymes need Mo?

In general, all Mo-enzymes catalyze the transfer of an oxygen atom to or from a substrate (Hille 2002). Each reaction, either reduction or oxidation, is also characterized by transfer of two electrons, which enforce the Mo atom to vary its oxidation state between IV and VI. Mo-enzymes present in eukaryotes are homodimeric proteins functioning only as a dimer, but not as a monomer. Some harbour an electron transport chain involving different prosthetic groups (FAD, heme or Fe–S, Moco) that are bound to separate domains identified on the enzyme's monomer. Fig. 1 shows the domain structure of the five plant Mo-enzymes where the domains are connected by regions highly variable in sequence thus obviously serving as interdomain hinge regions. It is obvious that these Mo-enzymes can be subdivided into two classes: the SO-class of Mo-enzymes is formed by SO and NR, and the XO oxidase (XO) family is represented by XDH, and AO, both classes sharing domains. We will later see that mARC forms a separate group.

Sulfite oxidase

SO (EC 1.8.3.1), the name-giving enzyme for the members of the SO-family of Mo-enzymes, catalyzes the oxidation from sulfite to sulfate (Eilers et al. 2001). The SO gene is widespread and highly conserved within the plant kingdom. SO possesses only Moco as redox center (Fig. 1) and—together with mARC—is the simplest Mo-enzyme found in eukaryotes. Oxygen serves as the terminal electron acceptor for plant SO and is reduced to hydrogen peroxide (Hänsch et al. 2006). The latter finding explains the peroxisomal localization of plant SO (Nowak et al. 2004) since excess hydrogen peroxide generated during sulfite oxidation can easily be eliminated by catalase. The physiological role of plant SO has been clarified only recently. As sulfite is a strong nucleophile that can react with a wide variety of cellular components, it was assumed that SO has a sulfite-detoxifying function and is required for removing excess sulfite from the cell. In support of this, Brychkova et al. (2007) and Lang et al. (2007) independently found that in comparison to wild-type plants SO-deficient plants are more susceptible to high concentrations of sulfite while SO-overexpressing plants are more tolerant

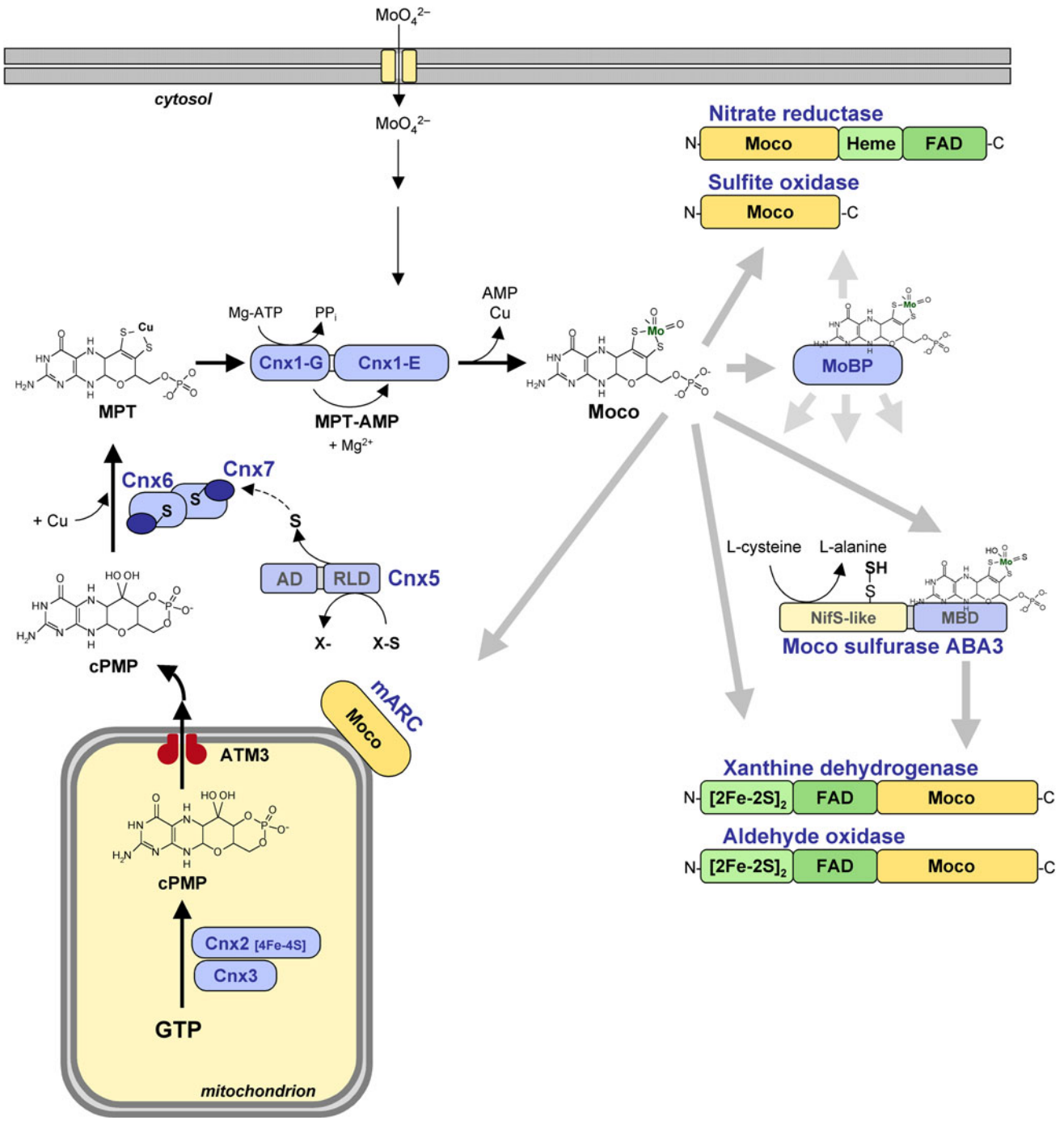

Fig. 1 Organization of biosynthesis, distribution, and maturation of Moco in plants. The basic steps of Moco biosynthesis are shown starting from the conversion of GTP to cPMP in the mitochondria, all subsequent steps proceed in the cytosol. Moco biosynthesis enzymes (named Cnx) and Moco-binding proteins (MoBP), are shown in *blue*. MPT-synthase, consisting of Cnx6 and Cnx7, is sulfurated by Cnx5, with the primary sulfur donor (X-S) mobilized by the rhodanese-like domain of Cnx5 (RLD) being unknown. The adenylation domain of Cnx5 (AD) is required for adenylation and activation of the small MPT synthase subunit Cnx7. It is assumed that copper (Cu) is inserted directly after dithiolene formation. The individual reactions of Cnx1 and its products (Moco, pyrophosphate PP_i, AMP, copper) are indicated. Moco can be either bound to a Moco-binding protein (MoBP), to the five Mo-enzymes NR, SO, XDH, AO and mARC, or to the Moco-binding C-terminal domain (MBD) of the Moco-sulfurase ABA3. The ABA3 C-terminus is the sulfuration platform for Moco. The NifS-like domain of ABA3 generates a protein-bound persulfide, which is transferred to Moco bound to its C-terminus which in turn exchanges non-sulfurated for sulfurated Moco. The domain structure of the Mo-enzyme monomers is given in the figure. It is obvious that the eukaryotic Mo-enzymes are evolutionary related to each other: XDH and AO form a pair, SO forms a pair with NR, and mARC arose from ABA3-MBD (modified after Bittner and Mendel 2010)

to excess sulfite. Under normal conditions however, the lack of SO activity in plants is not related to an obvious phenotype, suggesting that SO represents a salvage enzyme rather than a metabolic housekeeping enzyme (Hänsch et al. 2007).

Nitrate reductase

NR (EC 1.7.1.1., formerly EC 1.6.6.1) is a another member of the SO family and a key enzyme of nitrate assimilation, where it catalyzes the reduction of nitrate to nitrite in the cytosol. As NR thereby provides essential nitrogen metabolites to the plant, it is obvious that plants with a deficiency in NR are no longer nitrogen autotroph and depend on alternative nitrogen sources such as ammonium. The monomer of NR consists of three distinct domains (Fig. 1): the N-terminal domain associated with Moco, the central heme-binding cytochrome b_5 domain, and the C-terminal FAD-binding domain, whereby two such monomers form the active homodimeric enzyme. The domains are separated by solvent-exposed and protease-sensitive linker regions, called hinge I and hinge II. In plants hinge I, the linker between the cytochrome b_5 domain and the Moco domain, contains a conserved serine residue which mediates contact with a 14-3-3 protein when phosphorylated, subsequently leading to inhibition of enzyme activity (Kaiser and Huber 2001). The three domains form three redox centers catalyzing the transfer of electrons from the reductant NAD(P)H via FAD, heme, and Moco to nitrate.

In contrast to the reaction catalyzed by SO the process of nitrate reduction consumes rather than produces electrons deriving either from NADH or from NADPH. The crystal structures of SO (Schrader et al. 2003) and of the Moco domain of NR (Fischer et al. 2005) revealed a high degree of structural conservation between both proteins with only few differences in the boundaries of the enzymes. Substrate binding is proposed to be very similar among SO and NR due to three overall conserved residues in the actives sites. Yet, there are also important changes within the active site that are believed to determine substrate specificity and catalytic properties. These structures revealed that the Mo-domain can be further subdivided into a Moco-binding catalytic domain and a C-terminal domain representing the dimerization interface of holo-NR. It is remarkable that the Moco is completely buried inside the protein and a funnel-like structure leads from the surface of the protein to the active site.

While in terms of nitrogen assimilation nitrite is further reduced in the chloroplasts to ammonium by nitrite reductase, it can also be reduced to the signalling molecule nitric oxide (NO) by NR itself (Yamasaki and Sakihama 2000) As post-translational modification of NR also modulated the NO-production rates, it was concluded that NR is indeed a producer of active nitrogen species also in vivo (Wang et al. 2011).

Xanthine dehydrogenase

The members of the XO family are molybdo-flavoenzymes that catalyze the oxidative hydroxylation of a wide range of aldehydes and aromatic heterocycles. Plant XDH (EC 1.17.1.4., formerly EC 1.1.1.204) is a key enzyme of purine degradation where it oxidizes hypoxanthine to xanthine and xanthine to uric acid. The monomer can be subdivided into three distinct domains (Fig. 1): an N-terminal domain for binding of two iron-sulfur clusters of the [2Fe-2S]-type, a domain harbouring a FAD-binding site, and a C-terminal domain required for Moco-binding and dimerization. Electrons derived from substrate hydroxylation at the Mo-center go via the Fe-S clusters to the flavin cofactor. At the FAD site, the electrons are transferred either to NAD^+ to form NADH, or they are transferred to molecular oxygen to yield superoxide anions.

In addition to the hypoxanthine/xanthine-dependent production of reactive oxygen species (ROS) also NADH oxidase activity with simultaneous production of superoxide has been shown for plant XDH (Zarepour et al. 2010). Since this activity is very pronounced, this implies that XDH is an efficient producer of superoxide also in vivo, and that the enzyme may be involved in the regulation of the cellular NADH/NAD^+ balance. The ROS production of plant XDH might also be of physiological importance because increasing XDH activities and simultaneous ROS production were observed upon plant–pathogen interactions (Montalbini 1992a, b), hypersensitive response (Montalbini and Della Torre 1996), drought stress (Yesbergenova et al. 2005), virus infection (Silvestri et al. 2008) and natural senescence (Pastori and Rio 1997; Hesberg, et al. 2004). The subcellular localization of XDH is still not absolutely clear. Both, a cytosolic (Datta et al. 1991) and peroxisomal localization (Sandalio et al. 1988) was reported, a recent article localizes XDH to both the cytosol and peroxisomes (Corpas et al. 2008).

Aldehyde oxidase

AO proteins are cytoplasmic enzymes (EC 1.2.3.1) that catalyze the oxidation of a variety of aromatic and non-aromatic heterocycles and aldehydes, thereby converting them to the respective carboxylic acid. AO enzymes are very similar to XDH (Fig. 1) as they share a high degree of sequence homology, so that it is presumed that during

evolution AO has derived from XDH by gene duplication and neo-functionalization (Terao et al. 2001; Rodriguez-Trelles et al. 2003). The most prominent characteristics which distinguish AO from XDH enzymes have been found to concern the substrate binding at the Mo-center and binding of the physiological electron acceptor (Hille 2005). AO enzymes are strict oxidases that are unable to bind NAD^+ and exclusively use molecular oxygen as electron acceptor. Upon transfer of substrate-derived electrons to molecular oxygen, plant AO generates hydrogen peroxide (Yesbergenova et al. 2005).

The Arabidopsis genome harbors four AO genes, *AAO1–AAO4*, whose products form homodimers as well as heterodimers, thereby leading to altered substrate specificities of the respective isoenzymes. In 6-day-old seedlings, the gene products of *AAO1* and *AAO2* form three homo- and heterodimeric AO isoenzymes capable of producing indole-3-acetic acid (Akaba et al. 1999). The fact that IAA belongs to the auxin family of plant hormones suggests a possible physiological role of these AO enzymes in auxin biosynthesis during early stages of plant development. The AAO3 homodimer is characterized by a high preference for abscisic aldehyde as substrate (Seo et al. 2000a, b), which is the ultimate precursor of abscisic acid (ABA) that is involved in many aspects of plant growth and development, including seed maturation, dormancy, leaf senescence as well as adaptation to a variety of environmental stresses (Seo and Koshiba 2002; Verslues and Zhu 2005). Arabidopsis mutants with a deficiency in AAO3 therefore are characterized by reduced ABA levels accompanied by excessive water loss and a wilty phenotype, and also by retarded vegetative growth and reduced stress tolerance (Seo et al. 2000a, b). Recently, AAO3 protein levels have been shown to be regulated by ubiquitin-dependent degradation via the 26S-proteasome to prevent premature senescence by accumulation of ABA (Raab et al. 2009). This suggests that ABA-synthesizing AO proteins play a critical role also during the onset of senescence, which requires a tight control of AO and ABA levels.

Mitochondrial amidoxime reductase

In mammals, the mitochondrial amidoxime reducing component mARC was shown to catalyze the reduction of a variety of *N*-hydroxylated compounds, some of which are widely used as prodrugs and thus, are of pharmaceutical relevance (Havemeyer et al. 2006); Gruenewald, et al. 2008). All eukaryotic genomes known to encode proteins for Moco biosynthesis and Mo-enzymes, likewise encode two mARC proteins, suggesting that mARC proteins form an own small protein family (Wahl et al. 2010). All eukaryotic mARC proteins including the plant counterparts, are characterized by the presence of N-terminal extensions which predict a mitochondrial localization of these proteins. With an average molecular mass of $\sim$35 kDa and due to the fact that they bind Moco as the only prosthetic group, mARC protein are the smallest Mo-enzymes identified as yet. It is assumed that they play a detoxifying role in metabolism. In contrast to all other Mo-enzymes, eukaryotic mARC proteins do not exhibit enzymatic activity on their own but require other proteins like cytochrome b_5 and NADH/cytochrome b_5 reductase as electron transmitters and electron donors, respectively.

Molybdenum uptake into cells

How do Mo-enzymes acquire their catalytically important metal? Organisms take up Mo in the form of its molybdate anion. It requires specific uptake systems to scavenge molybdate in the presence of competing anions. In bacteria, high-affinity ABC-type transporters are described and in some bacteria specific molybdate-binding proteins are known with a capacity of up to eight anions (Pau and Lawson 2002) that store molybdate until further use by the cell. In contrast to bacterial molybdate homeostasis, eukaryotic molybdate transport is less well understood. Algae and higher plants are the only eukaryotes of which the molybdate uptake mechanisms have been unveiled recently. The protein MOT1 belonging to the large sulfate carrier family was shown to transport molybdate with ultra-high affinity (nanomolar k_M value) across cellular membranes (Baxter et al. 2008; Tejada-Jimenez et al. 2007; Tomatsu et al. 2007). Surprisingly, it was not found to reside in the plasma membrane. Contradictory reports localized it to the endomembrane system (Tomatsu et al. 2007) or to the mitochondrial envelope (Baxter et al. 2008). Both suggested subcellular locations are questionable as the insertion of Mo into the Moco-backbone takes place in the cytosol. A recent paper described the vacuole as an important molybdate store and provided functional evidence for a second molybdate transporter (MOT2) which was localized to the tonoplast (Gasber et al. 2011). This result is supported by a paper analyzing the proteome of the tonoplast where MOT2 was reported to be a tonoplast protein (Carter et al. 2004). Still the cellular importer for Mo is missing, but it is likely that additional transporters, not only in autotrophs but also in animals, will carry out this task. Here the novel transporter CrMOT2 found both in *Chlamydomonas reinhardtii* and humans (Tejada-Jimenez et al. 2011) is a good candidate to play this role. Further, it can be assumed that in addition to a possible high-affinity system molybdate could also non-specifically enter the cell

through the sulfate uptake system which was recently shown for a sulfate transporter (Fitzpatrick et al. 2008).

The Molybdenum cofactor

Once having entered the cell, Mo has to be attached to its cofactor scaffold thereby converting to Moco. Early work with mutants of *Nicotiana tabacum* (Mendel and Müller 1976) and the filamentous fungus *Aspergillus nidulans* (Cove and Pateman 1963) had postulated the existence of Moco. These genetic experiments revealed a novel mutant phenotype, namely the simultaneous loss of the two Mo-enzymes NR and XDH. Since Mo was the only common link between these two—otherwise very different—enzymes, it was suggested that both enzymes should share a common Mo-related cofactor, named Moco. Later, the Rajagopalan-group identified this cofactor as a tricyclic pteridine that coordinates the metal via a dithiolene group (Fig. 1, right upper part). Because of the unique nature of the pterin in Moco, the metal-free form of the cofactor is called molybdopterin or metal-containing pterin (MPT). The pterin structure of Moco is unique in nature and has probably been evolved in order to control and maintain the special redox properties of Mo. The task of the cofactor is to position the catalytic metal Mo correctly within the active center, to control its redox behaviour and to participate with its pterin ring system in the electron transfer to or from the Mo atom. The pterin with its several possible reduction states as well as different structural conformations could also be important for channeling electrons to other prosthetic groups (Kisker et al. 1997a, b). X-ray crystallographic analyses of Mo-enzymes revealed that the cofactor is not located on the surface of the protein, but it is buried deeply within the interior of the enzyme and a tunnel-like structure makes it accessible to the appropriate substrates (Kisker et al. 1997a, b; Fischer et al. 2005). During its life time, the Mo-enzyme does not liberate Moco. In vitro, however, one can remove Moco from the holoenzyme whereafter Moco loses Mo and undergoes rapid and irreversible loss of function due to oxidation (Rajagopalan 1996). The demolybdo-forms of Mo-enzymes are catalytically inactive. In bacteria, the Moco can also occur with a nucleotide attached via its phosphate group or in the form of two pterins coordinating one Mo (Schwarz et al. 2009).

Molybdenum cofactor biosynthesis

A mutational block of Moco biosynthesis leads to the loss of essential metabolic functions because all enzymes depending on Mo lose their activity, which ultimately causes death of the organism. In all organisms studied so far, Moco is synthesized by a conserved biosynthetic pathway that can be divided into four steps, according to the biosynthetic intermediates cPMP, MPT, adenlyated MPT, and Moco (Fig. 1). In eukaryotes always six gene products catalyzing Moco biosynthesis have been identified in plants (Mendel and Schwarz 2002), fungi (Millar et al. 2001) and humans (Reiss et al. 1998; Stallmeyer et al. 1999b). These genes are homologous to their counterparts in bacteria, and some but not all of the eukaryotic Moco biosynthesis genes are able to functionally complement the matching bacterial mutants. Genes and gene products were named in plants according to the *cnx* nomenclature (cofactor for nitrate reductase and xanthine dehydrogenase).

Biosynthesis in four steps

Step1 Moco biosynthesis starts in the mitochondria. GTP is transformed into the sulfur-free pterin compound cyclic pyranopterin monophosphate (cPMP), also known as precursor Z (Fig. 1), which is catalyzed by the proteins Cnx2 and Cnx3 in the mitochondrial matrix (Teschner et al. 2010). For its functioning, Cnx2 needs Fe-S clusters which are readily available in the mitochondrion. As all subsequent steps of Moco biosynthesis have been demonstrated to be localized in the cytosol, export of cPMP from the mitochondria into the cytosol is required to allow further processing to Moco. This task is fulfilled by the transporter protein Atm3, which is localized in the inner membrane of mitochondria and belongs to the family of ATP-binding cassette (ABC) transporters (Teschner et al. 2010). Surprisingly, Atm3 functions also in exporting a precursor compound essential for Fe-S cluster synthesis in the cytosol (Bernard et al. 2009).

Step 2 In the second stage sulfur is transferred to cPMP in order to generate the intermediate MPT (Fig. 1). This reaction is catalyzed by the enzyme MPT synthase, a heterotetrameric complex of two small (Cnx7) and two large (Cnx6) subunits that stoichiometrically converts cPMP into MPT. After MPT synthase has transferred the two sulfurs to cPMP, it has to be re-sulfurated by MPT-synthase sulfurase Cnx5 (Fig. 1) in order to reactivate the enzyme for the next reaction cycle of cPMP conversion. Cnx5 is a two-domain-protein that activates Cnx7 by adenylation (carried out by the Cnx5 N-terminal domain) followed by a sulfur transfer reaction (carried out by the Cnx5 C-terminal domain which has a rhodanese function). Cysteine is a likely candidate as final S-donor for the formation of MPT.

Step 3 After synthesis of the MPT moiety, the chemical backbone is built for binding and coordination of the Mo

atom. In the third step, therefore, Mo has to be transferred to MPT in order to form Moco, thus linking the molybdate uptake system to the MPT pathway. Mutants defective in this step produce MPT and can be partially repaired by growing them on high-molybdate medium (Mendel et al. 1981). In bacteria, this step is catalyzed by two proteins while during evolution to higher organisms these two proteins were fused to the two-domain protein Cnx1. The C-terminal Cnx1 domain (=Cnx1-G) adenylates MPT and forms the intermediate MPT-AMP that remains bound to Cnx1-G (Fig. 1). Most remarkably, the crystal structure of the Cnx1-G revealed a copper bound to the MPT dithiolate sulfurs (Kuper et al. 2004). Up to now the function of copper is unknown but it might play a role in sulfur transfer to cPMP, in protecting the MPT dithiolate from oxidation, and/or presenting a suitable leaving group for Mo insertion. The origin of copper is also unclear but it is reasonable to assume that it is transferred in vivo via the action of cytoplasmic chaperones thereafter binding to the dithiolate group of MPT just after the latter has been formed, i.e. at the end of step 2 of Moco biosynthesis. The copper is released after Mo has been inserted.

Step 4 In the final step of Moco biosynthesis (Fig. 1) MPT-AMP has to be converted into mature Moco: MPT-AMP is transferred to the N-terminal domain of Cnx1 (=Cnx1-E) thereby building a product-substrate channel. Cnx1-E cleaves the adenylate, releases copper and inserts Mo, thus yielding active Moco (Llamas et al. 2005). In vitro-studies with Cnx1-G-bound MPT-AMP revealed an inhibition of Moco synthesis in the presence of 1 μM $CuCl_2$, providing a link between Mo and copper metabolism (Llamas et al. 2006). Remarkably, the human homolog of Cnx1 is the protein Gephyrin (Stallmeyer et al. 1999b) which fulfills a dual role: it functions not only as Mo insertase but it serves also as anchor protein for inhibitory neuroreceptors in the postsynaptic membrane of neurons (Schwarz et al. 2009).

Distribution of molybdenum cofactor: storage and transfer

In higher organisms, the steps beyond Moco biosynthesis are less well known and, therefore, research focuses on Moco transport, allocation and insertion into apo-Mo-enzymes (Mendel and Schwarz 2011). Here one has to keep the cellular context in mind as Moco biosynthesis occurs to be micro-compartmentalized in a multiprotein-biosynthesis complex localized in the cytosol of the cell. After completion of biosynthesis, Moco either has to be allocated and inserted into the apo-Mo-enzymes, or Moco has to become bound to a carrier protein that protects and stores Moco until further use thus providing a way to buffer supply and demand of Moco. In bacteria, a complex of proteins synthesizing the last steps of Moco biosynthesis donates the mature cofactor to apo-enzymes assisted by enzyme-specific chaperones (Leimkuhler et al. 2011). Nearly each bacterial Mo-enzyme has a private chaperone available (Magalon et al. 2011); however, in higher organisms, no Mo-enzyme-specific chaperones have been found yet. Moco is highly unstable once liberated from proteins; it loses the Mo atom and undergoes rapid and irreversible loss of function due to oxidation. Therefore, it was assumed that Moco does not occur free but permanently protein-bound in the cell. A cellular Moco distribution system should meet two demands: (1) It should bind Moco subsequent to its synthesis, and (2) it should maintain a directed flow of Moco from the Moco donor Cnx1 to the Moco-dependent enzymes. The availability of sufficient amounts of Moco is essential for the cell to meet its changing demand of Mo enzymes.

A Moco-storage/carrier protein has been described for the green alga *Chlamydomonas reinhardtii* (a homotetramer that can hold four Moco molecules) donating Moco to NR. In *Arabidopsis thaliana*, recently a novel protein family was identified (Kruse et al. 2010) consisting of eight members (Fig. 1) that all can bind Moco and are therefore named Moco-binding proteins (MoBP). They seem to be involved in the cellular distribution of Moco. Obviously, land plants with differentiated organs need more MoBPs than a unicellular and motile alga. It is, however, still open whether these proteins represent the default-pathway for Moco-allocation to its users or whether it is only a buffer, and the default-way goes directly from Cnx1 to the appropriate apo-Mo-enzymes.

Insertion of molybdenum cofactor into Mo-enzymes

Among eukaryotes, nothing is known how FAD, iron-sulfur centers, and heme are bound to the Mo-enzymes. For Moco, however, the first crystallographic analyses of Mo-enzymes made evident that the cofactor is deeply buried within the holoenzyme so that Moco could only have been incorporated prior to or during completion of folding and dimerization of the apoprotein monomers. As there are three classes of Mo enzymes known in higher organisms (Hille et al. 2011) also differences in the insertion of Moco might be considered. For insertion of Moco into the target apo-enzymes either (still unknown) chaperone proteins would be needed or the Moco-carrier/binding proteins could become involved at this stage which has been shown for the *Arabidopsis* MoBP proteins. Once being introduced into the apoprotein, Mo is coordinated by additional ligands present in the active center of the respective Mo-enzyme. Like every other protein, also Mo-enzymes are

finally degraded after having fulfilled their tasks. One can assume that Moco is degraded as well because free Moco released from proteins is extremely labile and sensitive to oxidation.

Post-translational activation of AO and XDH

Eukaryotic Mo-enzymes are subdivided into two groups: NR and SO belong to the SO family of Mo-enzymes and are activated by insertion of Moco. XDH and AO, both members of the XO family of Mo-enzymes, require a final step of maturation during or after insertion of Moco, i.e. the addition of a terminal sulfido group to the Mo-center in order to gain enzymatic activity (Hille et al. 2011). This sulfur ligand is added by a separate enzymatic reaction catalyzed by the Moco-sulfurase ABA3 (Fig. 1). ABA3 is a homodimeric two-domain protein (Bittner et al. 2001) with its N-terminal domain sharing structural and functional homologies to bacterial cysteine desulfurases. In a pyridoxal phosphate-dependent manner, the N-terminal domain of ABA3 decomposes L-cysteine to yield alanine and elemental sulfur (Heidenreich et al. 2005), the latter being bound as a persulfide to a highly conserved cysteine residue of ABA3 (Fig. 1). The C-terminal domain of ABA3 shares a significant degree of similarity to the third class of Mo-enzymes in eukaryotes (=mARC proteins) and was shown to bind sulfurated Moco, which receives the terminal sulfur via intramolecular persulfide-transfer from the N-terminal domain (Wollers et al. 2008). It is likely that subsequent to Moco-sulfuration, ABA3 exchanges non-sulfurated for sulfurated Moco thus activating its target Mo-enzyme.

Under physiological aspects the terminal sulfuration step provides an efficient way of regulating the amount of active XDH and AO enzymes in the cell. The concentration of physiologically active compounds like hormones, as produced by plant AO, and ROS, as produced by the action of AO and XDH from many species, can rapidly be increased by changing the ratio of inactive and active XDH and AO molecules. In fact, a rapid induction of the *aba3* gene was found upon drought and salt stress in *A. thaliana* as well as upon ABA treatment (Xiong et al. 2001), thereby being consistent with the conditions required for induction of AO and XDH (Seo and Koshiba 2002; Hesberg et al. 2004; Yesbergenova et al. 2005).

Mo deficiency

The Mo content of plants is directly correlated to the bioavailability of Mo in the soil. The lower the soil pH, the less available is Mo thus causing Mo deficiency in plants (Mengel and Kirkby 2001). Mo-deficient plants develop a characteristic phenotype including lesions and altered morphology of leaves (referred to as "whiptail") that was first described by Arnon and Stout (1939) and later analysed in detail by Hewitt's group (Fido et al. 1977). In contrast, Mo toxicity in plants under most agricultural conditions is rare. There is an extensive literature about Mo nutrition in field-grown plants that was earlier reviewed (Kaiser et al. 2005).

Mo deficiency could also be caused by a mutation in the Mo-specific uptake system (Gasber et al. 2011). Knockout mutants in MOT1 showed a slightly altered growth pattern (Baxter et al. 2008; Tomatsu et al. 2007). As there are, however, several Mo transporters in a plant cell a severe phenotype has not yet been reported.

Finally, Mo deficiency can be caused by a defect in Moco biosynthesis which has dramatic consequences for the cell because pleiotropically all Mo-enzyme activities are lost or strongly reduced. The loss of which Mo-enzyme is most severe for the plant? (1) NR-mutants with complete loss of NR-activity are known since many years; the loss of NR activity is lethal if these mutants are cultured with nitrate as sole nitrogen source (Gabard et al. 1988; Müller and Mendel 1989). (2) The loss of SO has no phenotype provided the plants are not challenged to an atmosphere with high sulfur dioxide (Lang et al. 2007). (3) Also the loss of XDH1 has no lethal consequences although the phenotype of the plants is slightly, but not dramatically changed (F. Bittner and R. Mendel, unpublished). (4) For AO, only a mutation in AAO3 is communicated (Seo et al. 2000a, b). AAO3 is important for the conversion of abscisic aldehyde into ABA, hence its loss leads to a wilty phenotype which is severe for the survival of the plant. (5) A knockout in one of the two mARC proteins has no obvious phenotype (F. Bittner and R. Mendel, unpublished). (6) Moco-sulfurase (ABA3) deficiency was found to be basically ascribed to the reduction of ABA levels due to the lack of AO activities (Leon-Klooserziel et al. 1996; Xiong, et al. 2001) and hence a wilty phenotype. In summary, the complete loss of Moco as it occurs in *cnx*-mutants is lethal and leads to the death of plants when they are grown in soil. In cell culture, however, these mutants can be kept alive when grown on media with reduced nitrogen as N-source.

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References

Akaba S, Seo M, Dohmae N, Takio K, Sekimoto H, Kamiya Y, Furuya N, Komano T, Koshiba T (1999) Production of homo- and hetero-dimeric isozymes from two aldehyde oxidase genes of *Arabidopsis thaliana*. J Biochem (Tokyo) 126:395–401

Arnon DI, Stout PR (1939) Molybdenum as an essential element for higher plants. Plant Physiol 14:599–602

Baxter I, Muthukumar B, Park HC, Buchner P, Lahner B, Danku J, Zhao K, Lee J, Hawkesford MJ, Guerinot ML, Salt DE (2008) Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (MOT1). PLoS Genet 4:e1000004

Bernard DG, Cheng Y, Zhao Y, Balk J (2009) An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in Arabidopsis. Plant Physiol 151:590–602

Bittner F, Mendel RR (2010) Cell biology of molybdenum. Plant Cell Monogr 17:119–146

Bittner F, Oreb M, Mendel RR (2001) ABA3 is a molybdenum cofactor sulfurase required for activation of aldehyde oxidase and xanthine dehydrogenase in Arabidopsis thaliana. J Biol Chem 276:40381–40384

Bortels H (1930) Molybdän als Katalysator bei der biologischen Stickstoffbindung. Arch Mikrobiol 1:333–342

Brychkova G, Xia Z, Yang G, Yesbergenova Z, Zhang Z, Davydov O, Fluhr R, Sagi M (2007) Sulfite oxidase protects plants against sulfur dioxide toxicity. Plant J 50:696–709

Carter C, Pan S, Zouhar J, Avila EL, Girke T, Raikhel NV (2004) The vegetative vacuole proteome of *Arabidopsis thaliana* reveals predicted and unexpected proteins. Plant Cell 16:3285–3303

Corpas FJ, Palma JM, Sandalio LM, Valderrama R, Barroso JB, Del Rio LA (2008) Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. J Plant Physiol 165:1319–1330

Cove DJ, Pateman JA (1963) Independently segregating genetic loci concerned with nitrate reductase activity in *Aspergillus nidulans*. Nature 198:262–263

Datta DB, Triplett EW, Newcomb EH (1991) Localization of xanthine dehydrogenase in cowpea root nodules: implications for the interaction between cellular compartments during ureide biogenesis. Proc Natl Acad Sci USA 88:4700–4702

Eilers T, Schwarz G, Brinkmann H, Witt C, Richter T, Nieder J, Koch B, Hille R, Hansch R, Mendel RR (2001) Identification and biochemical characterization of Arabidopsis thaliana sulfite oxidase. A new player in plant sulfur metabolism. J Biol Chem 276:46989–46994

Fido RJ, Gundry CS, Hewitt EJ, Notton BA (1977) Ultrastructural features of molybdenum deficiency and whiptail in cauliflower leaves: effects of nitrogen source and tungsten substitution for molybdenum. Aust J Plant Physiol 4:675–689

Fischer K, Barbier GG, Hecht HJ, Mendel RR, Campbell WH, Schwarz G (2005) Structural basis of eukaryotic nitrate reduction: crystal structures of the nitrate reductase active site. Plant Cell 17:1167–1179

Fitzpatrick KL, Tyerman SD, Kaiser BN (2008) Molybdate transport through the plant sulfate transporter SHST1. FEBS Lett 582: 1508–1513

Gabard J, Pelsy F, Marion-Poll A, Caboche M, Saalbach I, Grafe R, Müller AJ (1988) Genetic analysis of nitrate reductase deficient mutants of *Nicotiana plumbaginifolia*: evidence for six complementation groups among 70 classified molybdenum cofactor deficient mutants. Mol Gen Genet 213:275–281

Gasber A, Klaumann A, Trentmann O, Trampczynska A, Clemens S, Schneider S, Sauer N, Feifer I, Bittner F, Mendel RR, Neuhaus HE (2011) Identification of an Arabidopsis solute carrier critical for intracellular transport and interorgan allocation of molybdate. Plant Biol (in press)

Gruenewald S, Wahl B, Bittner F, Hungeling H, Kanzow S, Kotthaus J, Schwering U, Mendel RR, Clement B (2008) The fourth molybdenum containing enzyme mARC: cloning and involvement in the activation of *N*-hydroxylated prodrugs. J Med Chem 51:8173–8177

Hänsch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR (2006) Plant sulfite oxidase as novel producer of H_2O_2: combination of enzyme catalysis with a subsequent non-enzymatic reaction step. J Biol Chem 281:6884–6888

Hänsch R, Lang C, Rennenberg H, Mendel RR (2007) Significance of plant sulfite oxidase. Plant Biol 9:589–595

Havemeyer A, Bittner F, Wollers S, Mendel R, Kunze T, Clement B (2006) Identification of the missing component in the mitochondrial benzamidoxime prodrug-converting system as a novel molybdenum enzyme. J Biol Chem 281:34796–34802

Heidenreich T, Wollers S, Mendel RR, Bittner F (2005) Characterization of the NifS-like domain of ABA3 from *Arabidopsis thaliana* provides insight into the mechanism of molybdenum cofactor sulfuration. J Biol Chem 280:4213–4218

Hesberg C, Hansch R, Mendel RR, Bittner F (2004) Tandem orientation of duplicated xanthine dehydrogenase genes from *Arabidopsis thaliana*: differential gene expression and enzyme activities. J Biol Chem 279:13547–13554

Hille R (2002) Molybdenum enzymes containing the pyranopterin cofactor: an overview. Met Ions Biol Syst 39:187–226

Hille R (2005) Molybdenum-containing hydroxylases. Arch Biochem Biophys 433:107–116

Hille R, Nishino T, Bittner F (2011) Molybdenum enzymes in higher organisms. Coord Chem Rev 255:1179–1205

Kaiser WM, Huber SC (2001) Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. J Exp Bot 52:1981–1989

Kaiser BN, Gridley KL, Brady JN, Phillips T, Tyerman SD (2005) The role of molybdenum in agricultural plant production. Ann Bot 96:745–754

Kisker C, Schindelin H, Pacheco A, Wehbi WA, Garrett RM, Rajagopalan KV, Enemark JH, Rees DC (1997a) Molecular basis of sulfite oxidase deficiency from the structure of sulfite oxidase. Cell 91:973–983

Kisker C, Schindelin H, Rees DC (1997b) Molybdenum-cofactor-containing enzymes: structure and mechanism. Annu Rev Biochem 66:233–267

Kruse T, Gehl C, Geisler M, Lehrke M, Ringel P, Hallier S, Hansch R, Mendel RR (2010) Identification and biochemical characterization of molybdenum cofactor-binding proteins from *Arabidopsis thaliana*. J Biol Chem 285:6623–6635

Kuper J, Llamas A, Hecht HJ, Mendel RR, Schwarz G (2004) Structure of molybdopterin-bound Cnx1G domain links molybdenum and copper metabolism. Nature 430:803–806

Lang C, Popko J, Wirtz M, Hell R, Herschbach C, Kreuzwieser J, Rennenberg H, Mendel RR, Hansch R (2007) Sulphite oxidase as key enzyme for protecting plants against sulphur dioxide. Plant Cell Environ 30:447–455

Leimkuhler S, Wuebbens MM, Rajagopalan KV (2011) The history of the discovery of the molybdenum cofactor and novel aspects of its biosynthesis in bacteria. Coord Chem Rev 255:1129–1144

Leon-Klooserziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevaart JAD, Koorneef M (1996) Isolation and characterization of abscisic acid-deficient *Arabidopsis* mutants at tow new loci. Plant J 10:655–661

Magalon A, Fedor JG, Walburger A, Weiner JH (2011) Molybdenum enzymes in bacteria and their maturation. Coord Chem Rev 255:1159–1178

Mendel RR (1992) The plant molybdenum cofactor (MoCo)—its biochemical and molecular genetics. In: Gresshoff PM (ed) Plant biotechnology and development—current topics in plant molecular biology. CRC Press, Boca Raton, pp 11–16

Mendel RR, Müller AJ (1976) A common genetic determinant of xanthine dehydrogenase and nitrate reductase in *Nicotiana tabacum*. Biochem Physiol Pflanzen 170:538–541

Mendel RR, Schwarz G (2002) Biosynthesis and molecular biology of the molybdenum cofactor (Moco). Met Ions Biol Syst 39:317–368

Mendel RR, Schwarz G (2011) Molybdenum cofactor biosynthesis in plants and humans. Coord Chem Rev 255:1145–1158

Mendel RR, Alikulov ZA, Lvov NP, Müller AJ (1981) Presence of the molybdenum-cofactor in nitrate reductase-deficient mutant cell lines of Nicotiana tabacum. Mol Gen Genet 181:395–399

Mengel K, Kirkby EA (2001) Principles of plant nutrition. Springer, Berlin

Millar LJ, Heck IS, Sloan J, Kana'n GJ, Kinghorn JR, Unkles SE (2001) Deletion of the cnxE gene encoding the gephyrin-like protein involved in the final stages of molybdenum cofactor biosynthesis in *Aspergillus nidulans*. Mol Genet Genomics 266:445–453

Montalbini P (1992a) Inhibition of hypersensitive response by allopurinol applied to the host in the incompatible relationship between *Phaseolus vulgaris* and *Uromyces phaseoli*. J Phytopathol 134:218–228

Montalbini P (1992b) Ureides and enzymes of ureide synthesis in wheat seeds and leaves and effect of allopurinol on *Puccinia recondita* f. sp. *tritici* infection. Plant Sci 87:225–231

Montalbini P, Della Torre G (1996) Evidence of a two-fold mechanism responsible for the inhibition by allopurinol of the hypersensitive response induced in tobacco by tobacco necrosis virus. Phys Mol Plant Pathol 48:273–287

Müller AJ, Mendel RR (1989) Biochemical and somatic cell genetics of nitrate reductase in *Nicotiana*. In: Wray JL, Kinghorn JR (eds) Molecular and genetic aspects of nitrate assimilation. Oxford University Press, Oxford, pp 166–185

Nowak K, Luniak N, Witt C, Wustefeld Y, Wachter A, Mendel RR, Hansch R (2004) Peroxisomal localization of sulfite oxidase separates it from chloroplast-based sulfur assimilation. Plant Cell Physiol 45:1889–1894

Pastori GM, Rio LA (1997) Natural senescence of pea leaves: an activated oxygen-mediated function for peroxisomes. Plant Physiol 113:412–418

Pau RN, Lawson DM (2002) Transport, homeostasis, regulation, and binding of molybdate and tungstate to proteins. Met Ions Biol Syst 39:39–74

Raab S, Drechsel G, Zarepour M, Hartung W, Koshiba T, Bittner F, Hoth S (2009) Identification of a novel E3 ubiquitin ligase that is required for suppression of premature senescence in Arabidopsis. Plant J 59:39–51

Rajagopalan KV (1996) Biosynthesis of the molybdenum cofactor. In: Neidhardt FC (ed) *Escherichia coli* and *Salmonella typhimurium*. ASM Press, Washington, DC, pp 674–679

Reiss J, Johnson JL (2003) Mutations in the molybdenum cofactor biosynthetic genes MOCS1, MOCS2, and GEPH. Hum Mutat 21:569–576

Reiss J, Cohen N, Dorche C, Mandel H, Mendel RR, Stallmeyer B, Zabot MT, Dierks T (1998) Mutations in a polycistronic nuclear gene associated with molybdenum cofactor deficiency. Nat Genet 20:51–53

Rodriguez-Trelles F, Tarrio R, Ayala FJ (2003) Convergent neofunctionalization by positive Darwinian selection after ancient recurrent duplications of the xanthine dehydrogenase gene. Proc Natl Acad Sci USA 100:13413–13417

Sandalio LM, Fernandez VM, Ruperez FL, del Rio LA (1988) Superoxide free radicals are produced in glyoxysomes. Plant Physiol 127:1–4

Schrader N, Fischer K, Theis K, Mendel RR, Schwarz G, Kisker C (2003) The crystal structure of plant sulfite oxidase provides insights into sulfite oxidation in plants and animals. Structure 11:1251–1263

Schwarz G, Mendel RR (2006) Molybdenum cofactor biosynthesis and molybdenum enzymes. Annu Rev Plant Biol 57:623–647

Schwarz G, Mendel RR, Ribbe MW (2009) Molybdenum cofactors, enzymes and pathways. Nature 460:839–847

Seo M, Koshiba T (2002) The complex regulation of ABA biosynthesis in plants. Trends Plant Sci 7:41–48

Seo M, Koiwai H, Akaba S, Komano T, Oritani T, Kamiya Y, Koshiba T (2000a) Abscisic aldehyde oxidase in leaves of Arabidopsis thaliana. Plant J 23:481–488

Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JA, Koornneef M, Kamiya Y, Koshiba T (2000b) The arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proc Natl Acad Sci USA 97:12908–12913

Silvestri S, Murphy AM, Buonaurio R, Carr JP (2008) Allopurinol, an inhibitor of purine catabolism, enhances susceptibility of tobacco to Tobacco mosaic virus. Virus Res 137:257–260

Stallmeyer B, Drugeon G, Reiss J, Haenni AL, Mendel RR (1999a) Human molybdopterin synthase gene: identification of a bicistronic transcript with overlapping reading frames. Am J Hum Genet 64:698–705

Stallmeyer B, Schwarz G, Schulze J, Nerlich A, Reiss J, Kirsch J, Mendel RR (1999b) The neurotransmitter receptor-anchoring protein gephyrin reconstitutes molybdenum cofactor biosynthesis in bacteria, plants, and mammalian cells. Proc Natl Acad Sci USA 96:1333–1338

Tejada-Jimenez M, Llamas A, Sanz-Luque E, Galvan A, Fernandez E (2007) A high-affinity molybdate transporter in eukaryotes. Proc Natl Acad Sci USA 104:20126–20130

Tejada-Jimenez M, Galvan A, Fernandez E (2011) Algae and humans share a molybdate transporter. Proc Natl Acad Sci USA 108:6420–6425

Terao M, Kurosaki M, Marini M, Vanoni MA, Saltini G, Bonetto V, Bastone A, Federico C, Saccone S, Fanelli R, Salmona M, Garattini E (2001) Purification of the aldehyde oxidase homolog 1 (AOH1) protein and cloning of the AOH1 and aldehyde oxidase homolog 2 (AOH2) genes. Identification of a novel molybdo-flavoprotein gene cluster on mouse chromosome 1. J Biol Chem 276:46347–46363

Teschner J, Lachmann N, Schulze J, Geisler M, Selbach K, Santamaria-Araujo J, Balk J, Mendel RR, Bittner F (2010) A novel role for Arabidopsis mitochondrial ABC transporter ATM3 in molybdenum cofactor biosynthesis. Plant Cell 22:468–480

Tomatsu H, Takano J, Takahashi H, Watanabe-Takahashi A, Shibagaki N, Fujiwara T (2007) An Arabidopsis thaliana high-affinity molybdate transporter required for efficient uptake of molybdate from soil. Proc Natl Acad Sci USA 104:18807–18812

Turnlund JR (2002) Molybdenum metabolism and requirements in humans. Met Ions Biol Syst 39:727–739

Verslues PE, Zhu JK (2005) Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress. Biochem Soc Trans 33:375–379

Wahl B, Reichmann D, Niks D, Krompholz N, Havemeyer A, Clement B, Meßerschmidt T, Rothkegel M, Biester H, Hille R, Mendel RR, Bittner F (2010) Biochemical and spectroscopic characterization of the human mitochondrial amidoxime reducing components hmARC-1 and hmARC-2 suggests the existence

of a new molybdenum-enzyme family in eukaryotes. J Biol Chem 285:37847–37859

Wang P, Du Y, Song CP (2011) Phosphorylation by MPK6: a conserved transcriptional modification mediates nitrate reductase activation and NO production? Plant Signal Behav (in press)

Wollers S, Heidenreich T, Zarepour M, Zachmann D, Kraft C, Zhao Y, Mendel RR, Bittner F (2008) Binding of sulfurated molybdenum cofactor to the C-terminal domain of ABA3 from Arabidopsis thaliana provides insight into the mechanism of molybdenum cofactor sulfuration. J Biol Chem 283:9642–9650

Xiong L, Ishitani M, Lee H, Zhu JK (2001) The arabidopsis los5/aba3 locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. Plant Cell 13:2063–2083

Yamasaki H, Sakihama Y (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. FEBS Lett 468:89–92

Yesbergenova Z, Yang G, Oron E, Soffer D, Fluhr R, Sagi M (2005) The plant Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase have distinct reactive oxygen species signatures and are induced by drought and abscisic acid. Plant J 42:862–876

Zarepour M, Kaspari K, Stagge S, Retmeiere R, Mendel RR, Bittner F (2010) Xanthine dehydrogenase AtXDH1 from *Arabidopsis thaliana* is a potent producer of superoxide anions via its NADH oxidase activity. Plant Mol Biol 72:301–310

Zhang Y, Gladyshev VN (2008) Molybdoproteomes and evolution of molybdenum utilization. J Mol Biol 379:881–899