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Review

Cell biology of molybdenum

Ralf R. Mendel *, Florian Bittner

Department of Plant Biology, Technical University of Braunschweig, Humboldtstrasse 1, 38106 Braunschweig, Germany

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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 diverse key reactions in the global carbon, sulfur and nitrogen metabolism. 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 eukaryotes, the most prominent Mo-enzymes are (1) sulfite oxidase, which catalyzes the final step in the degradation of sulfur-containing amino acids and is involved in detoxifying excess sulfite, (2) xanthine dehydrogenase, which is involved in purine catabolism and reactive oxygen production, (3) aldehyde oxidase, which oxidizes a variety of aldehydes and is essential for the biosynthesis of the phytohormone abscisic acid, and in autotrophic organisms also (4) nitrate reductase, which catalyzes the key step in inorganic nitrogen assimilation. All Mo-enzymes, except plant sulfite oxidase, need at least one more redox active center, many of them involving iron in electron transfer. 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 indespensable way. Moco as released after synthesis is likely to be distributed to the apoproteins of Mo-enzymes by putative Moco-carrier proteins. Xanthine dehydrogenase and aldehyde oxidase, but not sulfite oxidase and nitrate reductase, require the postranslational sulfuration of their Mo-site for becoming active. This final maturation step is catalyzed by a Moco-sulfurase enzyme, which mobilizes sulfur from L-cysteine in a pyridoxal phosphate-dependent manner as typical for cysteine desulfurases.

Keywords: Molybdenum cofactor; Sulfite oxidase; Nitrate reductase; Xanthine dehydrogenase; Aldehyde oxidase; Molybdenum cofactor deficiency

1. Introduction

Molybdenum (Mo) occurs in a wide range of metalloenzymes in bacteria, fungi, algae, plants and animals where it forms part of the active sites of these enzymes. In order to gain biological activity, Mo has to be complexed by a pterin compound thereby forming the molybdenum cofactor (Moco). In this article review, we will review and follow the way that Mo takes from uptake into the cell, via formation of the Moco and its storage, to the final modification of Moco and its insertion into apo-metalloenzymes. We will focus on eukaryotes because here our understanding of the cell biology of Mo is much more advanced. Most of this knowledge derives from studies in plants and humans which may be surprising—but yeast as model organism plays no role in Mo research as *Saccharomyces cerevisiae* is the only organism known to date that does not contain Mo-enzymes. Other yeasts like *Candida* and *Pichia*, however, need and synthesize Mo-enzymes.

It has been long known that the transition element Mo [1] 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. And also in soils, the molybdate anion is the only form of Mo that is available for plants and bacteria. Mo-containing enzymes hold key positions both in the biogeochemical redox

Abbreviations: AO, aldehyde oxidase; *A. thaliana*, *Arabidopsis thaliana*; Cnx1-E, N-terminal domain of Cnx1, homologous to *E. coli* MoeA; Cnx1-G, C-terminal domain of Cnx1, homologous to *E. coli* MogA; *C. rheinhardtii*, *Chlamydomonas rheinhardtii*; Cu, copper; Fe, iron; FAD, flavin adenine dinucleotide; MCP, molybdenum cofactor carrier protein; Mo, molybdenum; Moco, molybdenum cofactor; MPT, molybdopterin; NR, nitrate reductase; NO, nitric oxide; ROS, reactive oxygen species; SO, sulfite oxidase; XDH, xanthine dehydrogenase; XO, xanthine oxidase

* Corresponding author. Tel.: +49 531 391 5870; fax: +49 531 391 8128.
E-mail address: r.mendel@tu-bs.de (R.R. Mendel).

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cycles of carbon, nitrogen and sulfur on Earth [2] and in the metabolism of every the individual organism. To this end, more than 50 enzymes are known to contain Mo and most of them occur in bacteria while in eukaryotes only six were found [3]. 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 [4]. On the other side, unavailability of Mo is lethal for the organism. But even if Mo is available for the cell, it seems to be biologically inactive until it becomes complexed to form Moco thus gaining biological activity.

2. 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 molybdate transporters are described consisting of three protein components and requiring ATP-hydrolysis for operation. In some bacteria specific molybdate-binding proteins are known with a capacity of up to eight anions [5] that store molybdate until further use by the cell. In contrast to the well-studied molybdate transport and homeostasis mechanism in bacteria, eukaryotic molybdate transport is still poorly understood. In the alga *Chlamydomonas rheinhardtii* genetic evidence suggested the existence of a distinct molybdate uptake system [6] and very recently a molybdate transporter has been cloned from this organism (E. Fernandez, personal communication). In parallel, also in the model plant *Arabidopsis thaliana* a molybdate transporter has been cloned and characterized (T. Fujiwara, personal communication).

3. The molybdenum cofactor

Besides one other type of Mo-containing cofactor, Mo is bound to a unique tricyclic pterin compound named Moco (as shown on the bottom of Fig. 1). The other type of Mo-containing cofactor is found only once in nature namely in bacterial nitrogenase, forming the so-called FeMo-cofactor that consists of two partial cubanes ($MoFe_3S_3$ and Fe_4S_3) which are joined by three bridging sulfurs. Nitrogenase reduces atmospheric dinitrogen to ammonia under atmospheric pressure and temperature with concomitant hydrolysis of ATP. Nitrogenase is required for biological nitrogen fixation, which is an essential step in the nitrogen cycle in the biosphere, and a major contributor to the nitrogen available to many plants species like legumes. In contrast to nitrogenase all other Mo-containing enzymes characterized to this end contain the pterin-type cofactor [7]. For this reason, and as very recently a number of reviews about nitrogenase have been published (e.g., [8,9]) we will focus on the ubiquitously occurring Moco in this review.

Early work with mutants of the filamentous fungus *Aspergillus nidulans* and of the higher plant *Nicotiana tabacum* revealed a novel mutant phenotype, namely the simultaneous loss of the two Mo-enzymes nitrate reductase and xanthine dehydrogenase. 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. The elucidation of the chemical nature of Moco is based on the work of J. Johnson and K.V. Rajoagopalan. Their final description of Moco was confirmed by crystal structures of Mo-enzymes with the only exception that a third ring, a novel pyrano ring, is formed (Fig. 1). Due to the labile nature of Moco and its high sensitivity to oxidation most of the work was done by using degradation or oxidation products of the cofactor thereby revealing the pterin nature of Moco and its C6 substitution with a unique four-carbon side chain [10] that coordinates the metal via a dithiolene group (Fig. 1). Crystal structures of different Mo enzymes confirmed the core structure of Moco and helped to clarify the redox state of Moco [11]. Due to the formation of a third pyrano ring between the C3′ hydroxy group and the pterin C7 atom, a fully reduced hydrogenated pterin (tetrahydro state) is formed. 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 might also be important in channeling electrons from or to other prosthetic groups [12]. 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 [11,13]. Once Moco is liberated from the holoenzyme, it loses the Mo atom and undergoes rapid and irreversible loss of function due to oxidation [14]. The demolybdo-forms of Mo-enzymes are catalytically inactive.

4. 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. The identification of several genetic complementation groups among Moco-deficient mutants in a given organism and the conserved structure of Moco provided a basis to propose an evolutionary old multi-step biosynthetic pathway [15]. Already in pre-genomic times, a detailed mutant characterization contributed substantially to our understanding of the genetics and biochemistry of Moco biosynthesis in bacteria, plants, fungi and humans. Among eukaryotes, the molecular, biochemical and genetic analysis of Moco mutants was most advanced in higher plants. These results formed the basis to decipher Moco biosynthesis also in humans [16] where Moco deficiency is a severe genetic disease with fatal consequences for the affected individuals [17]. It turned out that the pathways of Moco biosynthesis showed many similarities in both organisms and therefore we will compare them whenever appropriate.

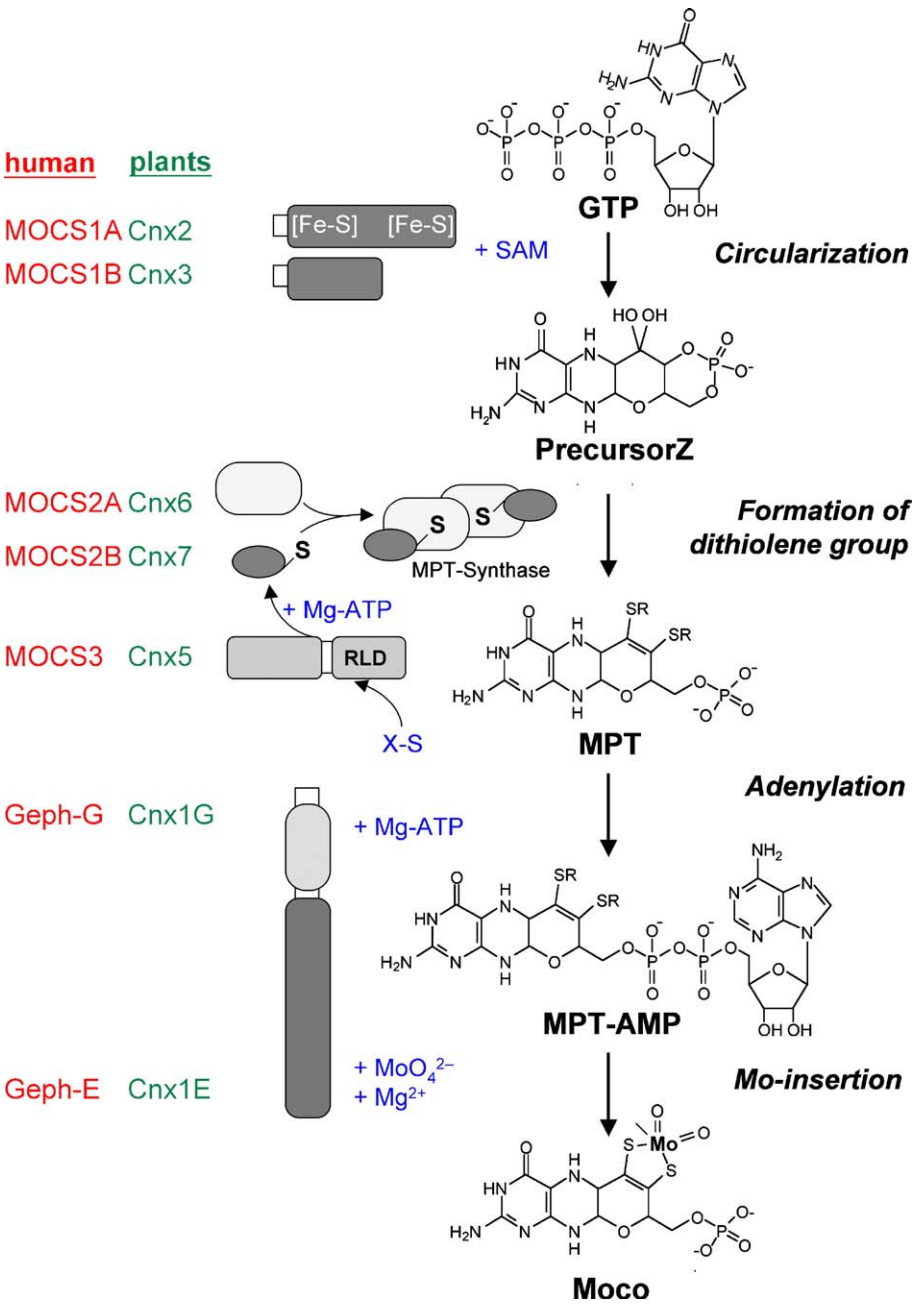

Fig. 1. Biosynthesis of eukaryotic molybdenum cofactor. The pathway of Moco synthesis can be divided into four steps, each being characterized by its main features as given in italics on the right side. For MPT and MPT-AMP, the ligands of the dithiolate sulfurs are indicated by an "R" as it is currently unknown at which state copper is bound to the dithiolate. Upon Mo insertion, it is also not clear how many oxo ligands are bound to the metal. Therefore, two Mo-oxo ligands are depicted and a third line indicates an additional ligand. The proteins from plants and humans catalyzing the respective steps are depicted and their names are given in green (plants) and red (humans). Functional properties like [Fe–S] clusters in Cnx2 and Mocs1A, the use of S-adenosyl methionine (SAM), adenylation and sulfuration of the small subunit of MPT synthase (Cnx7 and Mocs2B, respectively) are indicated. All substrates/co-substrates are indicated in blue. The in vivo source of sulfur (X–S) for Cnx5 and Mocs3 is not known yet. Steps three and four in plants and humans are catalyzed by the individual domains of Cnx1 (G and E) or Gephyrin (G and E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 precursor Z, MPT, adenlyated MPT, and Moco (Fig. 1). In eukaryotes always six gene products catalyzing Moco biosynthesis have been identified in plants [18], fungi [19] and humans [20–22]. 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 (*c*ofactor for *n*itrate reductase and *x*anthine dehydrogenase) introduced for the fungal mutants with the mutants labeled by letters (cnxA-F) and the cDNAs labeled by numbers (*cnx*1–3, *cnx*5–7). For human Moco synthetic genes, a different MOCS (molybdenum cofactor synthesis) nomenclature has

been introduced [20]. We will now discuss the individual steps of Moco biosynthesis.

4.1. Step 1: conversion of GTP into precursor Z

During the first stage, a guanosine derivative (probably GTP) is transformed into a sulfur-free pterin compound, the precursor Z, possessing already the Moco-typical four carbon side chain (Fig. 1). In comparison to Moco and MPT, precursor Z is the most stable intermediate with an estimated half life of several hours at a low pH [23]. Mass spectrometry and 1 H NMR recently revealed that precursor Z already possesses a fully reduced tetrahydropyranopterin structure and is predominantly hydrated at the C1′ position resulting in a geminal diol [24]. GTP labeling studies and NMR demonstrated that each carbon atom of the ribose and of the guanine ring are incorporated into precursor Z [25,26]. The detailed mechanism of this reaction step remains unclear, yet hypothetical multistep-reactions have been suggested [25,26]. In all organisms, the conversion of GTP to precursor Z is catalyzed by two proteins, one of them (Cnx2 in plants, MOCS1A in humans) belonging to the superfamily of S-adenosylmethionine-dependent radical enzymes [27]. Members of this large family catalyze the formation of protein and/or substrate radicals by reductive cleavage of SAM by a [4Fe–4S] cluster [28]. Both, Cnx2 and MOCS1A, have N-terminal extensions carrying putative targeting motifs for organellar transport.

4.2. Step 2: synthesis of molybdopterin

In the second stage, sulfur is transferred to precursor Z in order to generate MPT. This reaction is catalyzed by the enzyme MPT synthase, a heterotetrameric complex of two small and two large subunits that stoichiometrically converts precursor Z into MPT. The sulfur is bound to the C-terminus of the small subunit as thiocarboxylate. Due to the fact that each small subunit of MPT synthase carries a single sulfur atom, a two-step mechanism for the formation of the MPT dithiolate has been proposed, which involves the formation of a mono-sulfurated intermediate [29,30]. After MPT synthase has transferred the two sulfurs to precursor Z, it has to be re-sulfurated by the MPT-synthase sulfurase in order to reactivate the enzyme for the next reaction cycle of precursor Z conversion. This resulfuration is catalyzed by plant Cnx5 or human MOCS3, respectively, involving an adenylation of MPT synthase followed by sulfur transfer [31,32]. Cnx5 and MOCS3 are two-domain proteins consisting of a N-terminal domain responsible for adenylating MPT synthase and a C-terminal rhodanese-like domain where the sulfur is bound to a conserved cysteine in form of persulfide [32]. The identity of the donor for the reactive mobile sulfur is as yet unknown, but a redundant function of different persulfide-generating systems is possible [33].

4.3. Step 3: adenylation of molybdopterin

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. In bacteria, this step is catalyzed by two proteins while during evolution to higher organisms these two proteins were fused to a two-domain protein. Early it was assumed that one domain should be essential for generating an activated form of Mo that is incorporated by the other domain into bound MPT [34,35]. But only recently the exact mechanism was uncovered in plants where the protein Cnx1 is catalyzing this step [36]. The C-terminal Cnx1 domain (=Cnx1-G) was known to tightly bind MPT [37]. Yet, its crystal structure [38] revealed an unexpected finding: a novel reaction intermediate, adenylated MPT (MPT-AMP) (Fig. 1), was found. Subsequently, it was demonstrated that Cnx1-G adenylates MPT in a Mg^{2+}- and ATP-dependent way and forms MPT-AMP that remains bound to Cnx1-G [36].

4.4. Step 4: molybdenum insertion and crosstalk to copper metabolism

The crystal structure of the Cnx1-G revealed another unexpected finding, namely a copper bound to the MPT dithiolate sulfurs, whose nature was confirmed by anomalous scattering of the metal. In both structures the copper atom shows tetragonal coordination with two waters as additional ligands in the MPT-bound state, while one of these waters is replaced by a histidine in the MPT-AMP-bound structure. Up to now the function of this novel MPT ligand is unknown but copper might play a role in sulfur transfer to precursor Z, in protecting the MPT dithiolate from oxidation, and/or presenting a suitable leaving group for Mo insertion. The origin of this copper is still unclear but it is reasonable to assume that it binds to the enedithiolate group just after the latter has been formed, i.e., at the end of step 2 of Moco biosynthesis.

In the final step of Moco biosynthesis 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 that cleaves the adenylate, releases copper and inserts Mo, thus yielding active Moco. We found that MPT adenylate was hydrolyzed in a molybdate-dependent way [39]. This reaction was coupled to the metal exchange reaction where bound copper was released and Mo was transferred to MPT thus yielding mature Moco (Figs. 2 and 3). As copper is always found in a protein-bound state it might be that Cnx1 interacts with a copper chaperone when the metal is released during Mo insertion. Using a split-ubiquitin based two-hybrid approach with Cnx1 as bait, a copper chaperone homolog has been recently identified (J. Winking, R. Mendel, G. Schwarz, unpublished data).

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 [38]. Copper inhibition of Moco synthesis can be explained by inhibition of the Mg-dependent Mo insertion reaction. The latter is supported by the suppression of copper inhibition

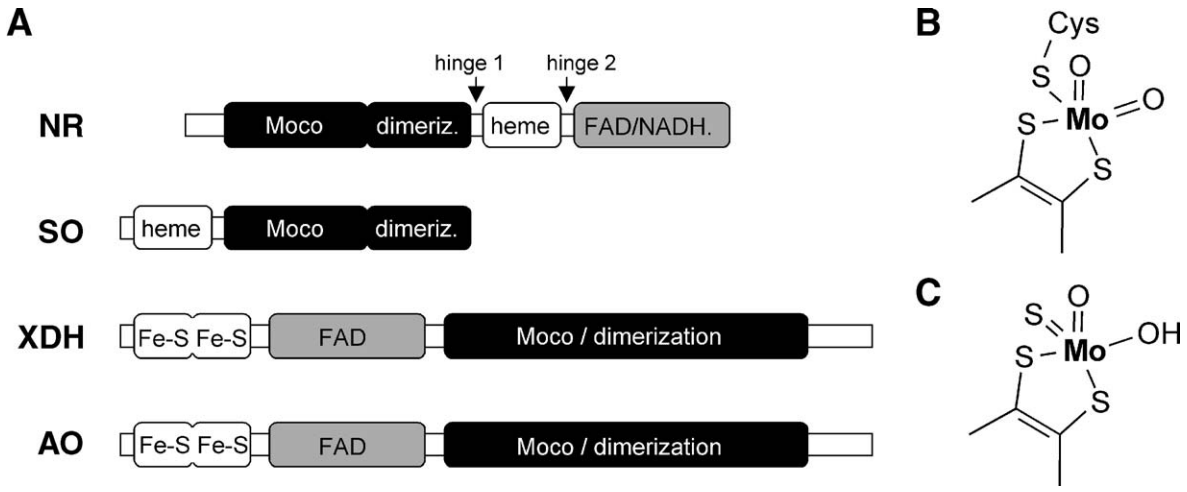

Fig. 2. Domain structure of eukaryotic molybdenum-enzymes (A). SO as depicted represents the animal form; SO from plants (not shown) is lacking the heme domain. Structure of the molybdenum center in enzymes of the SO/NR (B) and XDH/AO family (C).

with equimolar amounts of Cnx1-E and is in line with the known copper inhibition of pyrophosphatases [40]. Our finding implies that Moco deficiency might occur when cellular copper concentrations are increased, as seen in individuals affected with Wilson's disease [41], where copper accumulates in liver and brain, resulting in severe damage to both organs. Some of the symptoms may be attributed to an underlying Moco deficiency. However, this copper-Mo antagonism is opposite to the previously known Mo-copper antagonism (particularly in cattle), where increased molybdate causes copper deficiency owing to the formation of copper-chelating thiomolybdate complexes [42]. Further, also copper shortage should be detrimental for Moco biosynthesis. Therefore analysis of patients affected with Menke's disease, where copper transfer is impaired, could shed further light onto the link between Mo and copper metabolism.

5. Storage of the molybdenum cofactor

After synthesis, Moco has to be incorporated into the appropriate apo-enzyme. As Moco is labile and oxygen-sensitive [43] it was assumed that there is no free Moco occurring in the cell, rather it was suggested that Moco should be transferred immediately after biosynthesis to the apo-Mo-enzyme or that it could be bound to a carrier protein that protects and stores Moco until further use. The availability of sufficient amounts of Moco is essential for the cell to meet its changing demand for synthesizing Mo enzymes, therefore, the existence of a Moco carrier protein (MCP) would provide a way to buffer supply and demand of Moco. Among eukaryotes, first in the green alga *C. rheinhardtii* a MCP was described [44]. Later it was purified and a 16 kDa protein identified which was able to bind and protect Moco against oxidation [45,46]. *C. rheinhardtii* MCP forms a homotetramer (Fig. 3) in solution and is homologous to bacterial proteins with unknown function that contain a predicted nucleotide-binding Rossman fold. Among higher plants, proteins with homologies to *C. reinhardtii* MCP are found that are classified as lysine decarboxylase-like proteins forming a multi-gene family in *A. thaliana* with nine conserved members. The crystal structures of two of these proteins turned out to be highly similar to the crystal structure of *C. rheinhardtii* MCP (K. Fischer, G. Schwarz, unpublished data).

6. Insertion of the molybdenum cofactor into molybdenum enyzmes

Insertion of Moco into Mo-enzymes is not understood. Using a defined in vitro-system it was shown that human apo-sulfite oxidase can directly incorporate Moco [33]. However, for insertion of Moco into the target apo-enzymes as it occurs in the living cell either (still unknown) chaperone proteins would be needed or the Moco carrier proteins could become involved at this stage. For some bacterial Mo-enzymes, system-specific chaperones are required for Moco insertion and protein folding, e.g., NarJ for *E. coli* nitrate reductase [47] and XDHC for xanthine dehydrogenase from *Rhodobacter capsulatus* [48] and *Comamonas acidovorans* [49].

7. Crosslink to neuroreceptor anchoring

In plants, Cnx1 catalyzes the activation of MPT followed by insertion of Mo. The primary structure of Cnx1 shows striking homologies to the mammalian protein Gephyrin that was first described as a neuroreceptor anchor protein linking glycine receptors in the postsynaptic membrane to the subcellular cytoskeleton. The crystal structure of Gephyrin G domain is extremely similar to its plant homolog [50]. However, the orientation of the N-terminal and C-terminal domains is reversed in Gephyrin. Gephyrin is thought to be an instructive molecule for the formation of glycinergic synapses [51] and its expression was shown to be essential for the postsynaptic aggregation of glycine receptors [52].

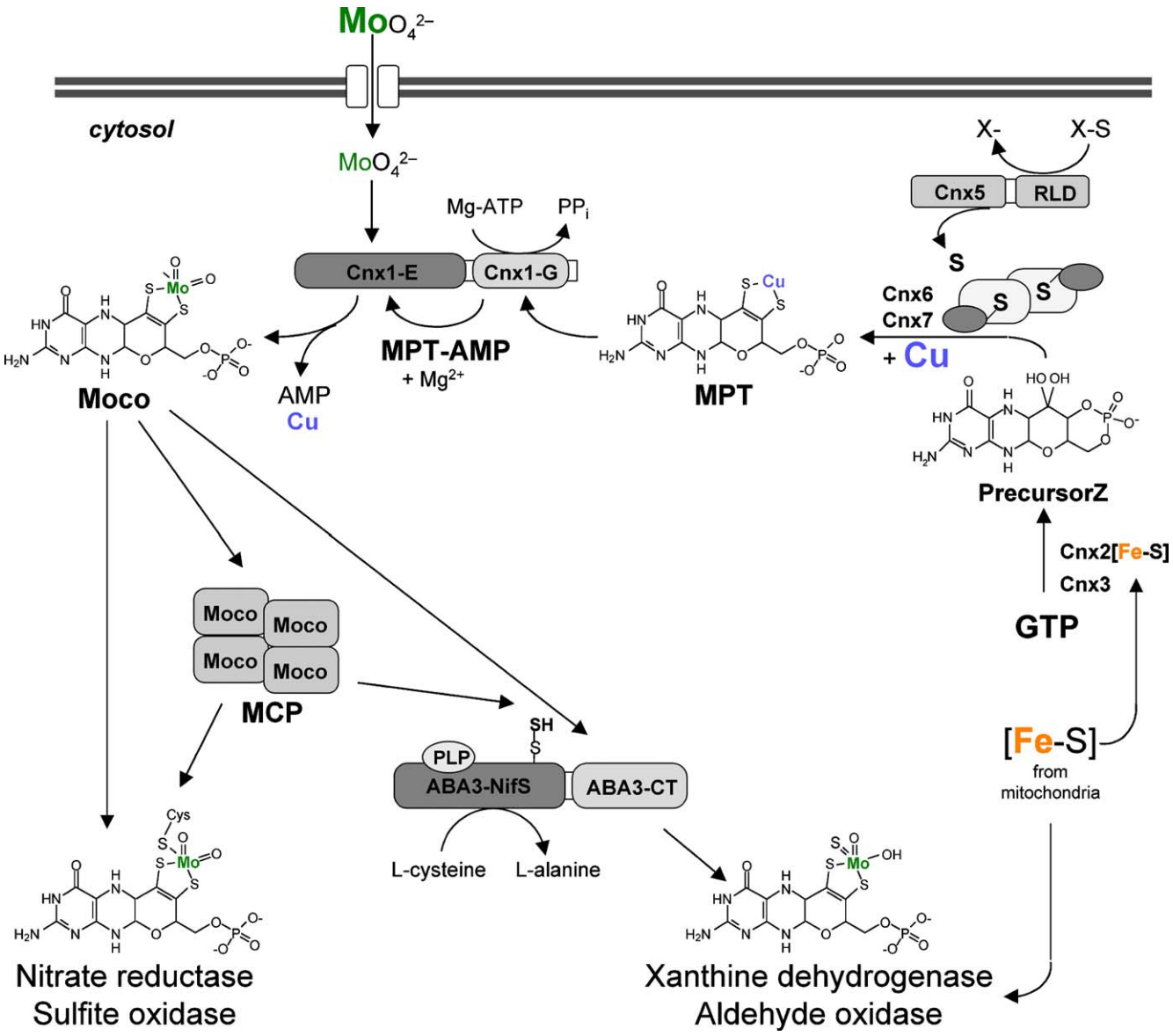

Fig. 3. Organization of biosynthesis, distribution, and maturation of Moco in plant cells. The basic steps of Moco biosynthesis are shown starting from GTP to Moco, including Moco biosynthesis enzymes. The dependence of Cnx2 on [Fe–S] from mitochondria is indicated. MPT-synthase, consisting of Cnx6 and Cnx7, is sulfurated by Cnx5, with the primary sulfur donor of Cnx5 (X–S) being unknown. It is assumed that copper (Cu) is inserted directly after dithiolene formation. The individual reactions of Cnx1 and its products (Moco, pyrophosphate, AMP, copper) are indicated. Due to subcellular localization and functional homology to human Gephyrin Cnx1 is believed to be in close proximity to the plasmamembrane where it might interact with the molybdate transporter. Mature Moco can be either bound to a Moco carrier protein (MCP), to NR and SO, or to the ABA3 protein. ABA3 is known to generate a protein-bound persulfide, which is the source of the terminal sulfur ligand of Moco in enzymes of the XDH/AO family. Like Cnx2, XDH and AO also depend on [Fe–S] from mitochondria. The different metals (Mo, Fe, and Cu) are indicated by different colours. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Based on the homology of Gephyrin to Cnx1 we suggested an additional function of Gephyrin in Moco biosynthesis, which was demonstrated by the following experiments. Recombinant Gephyrin binds MPT with high affinity and heterologous expression of Gephyrin could restore Moco biosynthesis in *E. coli*, plants and the murine cell line L929 [22]. In addition, *gephyrin* knockout-mice did not only show the expected absence of synaptic glycine receptor clustering, but also developed symptoms identical to those of Moco deficiency [53] where no SO activity could be detected. Recently, we identified a *gephyrin* gene deletion in a patient with symptoms typical for Moco deficiency [54]. Biochemical studies with the patient's fibroblasts demonstrated that Gephyrin catalyzes the insertion of Mo into MPT suggesting that this form of Moco deficiency might be curable with high doses of molybdate. It is obvious that Gephyrin combines two different functions: (1) a biosynthetic activity in Moco formation, and (2) a structural role in receptor clustering. The latter function is evolutionary younger and hence must have been recruited from the older in primary metabolism. The observation that differentially spliced transcripts of *gephyrin* can be found not only in brain and spinal cord, but also in liver, kidney, heart and lung [55] raises the possibility that differential splicing of *gephyrin* results in the modulation of its functionality.

8. Micro-compartmentalization and cytoskeleton binding

Not only Gephyrin, but also its plant homologue Cnx1 exhibits functional properties that are distinct from Moco biosynthesis. Based on the observed cytoskeleton binding of Gephyrin, a binding of Cnx1 to actin filaments, exclusively mediated by the E domain, could be demonstrated [35]. Also Gephyrin binds to actin filaments via its E-domain [56], and this domain also interacts with profilin, a major modulator of actin polymerization [57].

Finally, Cnx1 is essential for stabilizing the newly formed Moco [58]. What could be the functional significance of cytoskeleton binding of Cnx1 in terms of Moco biosynthesis? We assume that during evolution it became important to facilitate product-substrate flow, which could result in micro-compartmentalization of a hypothetical Moco biosynthetic multi-enzyme complex ensuring the fast and protected transfer of the labile intermediates within the reaction sequence from GTP to Moco. Therefore anchoring to cellular structures like the cytoskeleton might help organizing and stabilizing such a biosynthetic machinery and would bring it close to the molybdate transporter providing the metal for Moco synthesis.

In humans, Moco biosynthesis is very similar to the pathway described for higher plants. Surprisingly, differences were found on the gene level. Like in all other organisms, two proteins are involved in the conversion of GTP to precursor Z (step 1), however these two proteins are encoded by only one gene (*mocs1*) in human [59]. The corresponding transcript is bicistronic with two consecutive reading frames separated by a stop codon. The first reading frame encodes for MOCS1A, the second one for MOCS1B, and for both proteins human patients were identified [60]. Recently, further transcripts of the *mocs1* gene were found [61] that are spliced in order to bypass the normal termination codon of *mocs1A*.

The human system became even more fascinating when we found that also the two subunits of human MPT synthase (step 2) were encoded by only one gene, named *mocs2* [21]. On the bicistronic messenger RNA, the first reading frame codes for the small subunit MOCS2A and the second one for the large subunit MOCS2B. Both reading frames do overlap and exhibit a frameshift of + 1 for *mocs2B*. Again here, human patients were identified for both proteins [62] thus confirming their functional role. In both cases of bicistronic expression, always the first of the two encoded proteins shows a functionally important Gly–Gly motif at its C-terminus. Bicistronicity would ensure colinear expression and implicates vicinity of the newly synthesized and interacting proteins. Such a microcompartmentalization is certainly advantageous for low substrate concentrations like in Moco biosynthesis. Yet, it remains enigmatic why in the human Moco biosynthetic pathway two times the extremely rare case of bicistronicity is found while in higher plants the corresponding genes are widely separated and like in the case of the step 1 genes are even located on different chromosomes. This is surprising because in particular the plants would need vicinity of the newly synthesized and interacting proteins because here we encounter a strong cytoplasmic streaming.

9. Molybdenum cofactor biosynthesis and the evolution of ubiquitin

During evolution, not only glycine receptor anchoring lend support from Moco biosynthesis by recruiting Gephyrin, but also the development of ubiquitin used Moco biosynthesis as toolbox. When comparing crystal structures, the small subunit of MPT synthase from *E. coli* revealed an unexpected high structural similarity to ubiquitin including the terminal double glycine motif. In addition, the bacterial MPT synthase sulfurase MoeB that is essential for activating the small subunit of MPT synthase, is homologous in its entire region to the N-terminal part of the ubiquitin-activating enzyme UbA1 from *Saccharomyces cerevisiae* [63,64]. Further, the ATP-dependent mechanism by which both ubiquitin and the small subunit of MPT synthase are activated, is identical. These similarities suggest that the ubiquitin dependent protein degradation evolved from the evolutionary older pathway of Moco biosynthesis.

10. Molybdenum cofactor deficiency and therapy

For higher organisms like man and plants, a shortage of Mo in nutrition or a mutational block of the cellular ability to use Mo – i.e., to synthesize MPT, to take up Mo into the cell or to bind it to MPT – leads to the loss of essential metabolic functions because all enzymes needing Mo lose their activity at the same time. In humans, a combined deficiency of Mo-enzymes was first described by Duran et al. [65]. Babies born with this defect show feeding difficulties, severe and progressive neurologic abnormalities, and dysmorphic features of the brain and head. So far, disease-causing mutations have been identified in three of the four known Moco-synthetic human genes: *mocs1*, *mocs2* and *gephyrin* [16]. To this end, more than 100 cases have been diagnosed, and approximately 50 unrelated families have been analyzed at the molecular level [16]. The incidence, however, is estimated to be higher than these numbers would indicate, as it is likely that many cases remain unrecognized. The clinical symptoms may result from the deficiency of SO that protects the organism, in particular the brain, from elevated levels of toxic sulfite [17].

To this end, no therapy is available to cure the symptoms of this disease. Moco deficiency cannot be treated by supplementation with the cofactor. Moco is extremely unstable outside the protecting environment of an apo-Mo-enzyme. Its half-life is only a few minutes in aqueous solutions with neutral pH [66]. In addition, no chemical synthesis of Moco or any of its intermediates has been successful so far, which hampers its large-scale production for therapeutic use. However, very recently, we have developed a model that could lead to the cure of Moco-deficiency. Genetic analyses of patients showed that most of them had defects in the first step of Moco biosynthesis, i.e., the conversion of GTP to precursor Z [60]. Our idea was to treat patients of this class with the missing intermediate precursor Z because the steps subsequent to precursor Z formation are not affected by the mutation and should facilitate the synthesis of Moco. Precursor Z is more stable than Moco itself and has an identical structure in all

organisms. Thus, it was overproduced in the bacterium *E. coli* and purified. MOCS1 knockout-mice with a block in the first step of Moco biosynthesis were created bearing a genetic defect identical to the human patients [67]. Similar to humans, heterozygous mice displayed no symptoms, but homozygous Moco-deficient animals displayed symptoms resembling those of the human deficiency state and they died within ten days after birth. Due to the mutation, no MPT or active Moco was detectable, and consequently all Mo-enzyme activities were absent.

Repeated injections of precursor Z into MOCS1-deficient mice resulted in a dose-dependent extension of life span [68]. MPT levels and Mo-enzyme activities were partially restored. Stopping precursor Z treatment at any time resulted in a progressive reduction of MPT levels and Mo-enzyme activities and death of the animal 10–15 days after receiving the last injection. Injection of precursor Z into these mice every second day normalized their symptoms, they reached adolescence and were fertile [68]. It remains to be seen whether delayed onset of the described therapy will still allow reversal of neurological damage. As a next step, scaling up of precursor Z production is in progress in order to have sufficient amounts available for clinical trials.

11. Molybdenum enzymes

Mo-enzymes are required for diverse key reactions in the global carbon, sulfur and nitrogen metabolism, and up to now more than 50 different mononuclear Mo-enzymes have been found in nature, mostly from bacterial origin. Among those dissimilatory and respiratory nitrate reductase, DMSO reductase, formate dehydrogenase and TMAO reductase are prominent.

In contrast, only a limited number of Mo-enzymes is present in eukaryotes that can be subdivided into two classes: the xanthine oxidase (XO) family is represented by xanthine dehydrogenase (XDH), aldehyde oxidase (AO), pyridoxal oxidase and nicotinate hydroxylase, and the SO class of Mo-enzymes is formed by sulfite oxidase (SO) and nitrate reductase (NR). While pyridoxal oxidase and nicotinate hydroxylase were exclusively found in *Drosophila melanogaster* [69] and *Aspergillus nidulans* [70], respectively, XDH, AO, and SO are typical for all eukaryotes analyzed so far. As NR is required for nitrate assimilation this enzyme is only present in autotrophic organisms like plants, algae and fungi. In general, reactions catalyzed by Mo-enzymes are characterized by the transfer of an oxygen atom, ultimately derived from or incorporated into water, to or from a substrate in a two-electron redox reaction [12,71] in which the oxidation state of Mo ranges between IV and VI. Below we will focus on the eukaryotic Mo-enzymes with their specific functions, physiological roles and distribution within the cell.

12. 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. XDH (EC 1.1.1.204) is a key enzyme of purine degradation and oxidizes hypoxanthine to xanthine and xanthine to uric acid by simultaneous release of electrons from the substrate. The enzyme is active as a homodimer composed of two identical subunits of approximately 150 kDa, each being subdivided into three distinct domains (Fig. 2A): an N-terminal domain with a size of 20 kDa for binding of two [2Fe–2S] clusters, a 40 kDa domain harboring 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 Fe–SII and Fe–SI 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. Animal XDH [72], but not plant XDH [73], can also form hydrogen peroxide. Both electron acceptors can be used by XDH, but not by the oxidase form (XO), which in some organisms derives from reversible [74] or irreversible [75] conversion of XDH. XO exclusively transfers electrons to molecular oxygen with concomitant production of superoxide. 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 the mammalian [76] as well as for the plant enzyme [73]. Therefore, XDH enzymes are discussed not only to decompose purines but also to have additional physiological functions in ROS metabolism. Human XDH is discussed to be a pathogenic agent in many forms of ischaemia–reperfusion injury and might also be involved in inflammatory signal transduction [77,78]. 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 [79,80], hypersensitive response [81], drought stress [73] and natural senescence [82,83].

In humans, XDH activity is high in the liver and the lung [77,84], while in mice and rats highest XDH activity was found in the first part of the intestinal tract [84, 85]. In addition, XDH from mice was shown to be present also in the lactating mammary epithelium where it obviously plays an important role in enveloping milk fat droplets with the apical plasma membrane prior to secretion from the lactating mammary gland [86,87]. Thus, besides its enzymatic function XDH plays also a role as membrane-associated structural protein in the secretion milk fat droplets.

The subcellular localization of both, animal and plant XDH, is still not absolutely clear. While cytosolic localization in endothelian cells of bovine tissue [88]) and in rat hepatocytes [89] was shown on the one hand, ultrastructural studies with rat hepatocytes identified XDH activity in the peroxisomes [90,91]. Also for plant XDH cytosolic [92] and peroxisomal localization [93] was reported. Yet it should be mentioned that neither in two-dimensional polyacrylamide gelelectrophoresis with highly pure peroxisomes nor by use of the AraPerox database [94], specifically designed for identifying peroxisomal proteins from *A. thaliana*, XDH from *A. thaliana* was detected in this compartment.

13. 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 enzymes (Fig. 2A) as they share a high degree of sequence homology, have nearly identical molecular mass, bind the same cofactors, form dimers, and also act as hydroxylases. AO enzymes can be aligned along their entire length with XDH enzymes and phylogenetic analysis has shown that AO proteins have derived from XDH after ancient gene duplications [95]. 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 [96]. AO enzymes are strict oxidases that are unable to bind NAD^+ and exclusively use molecular oxygen as electron acceptor. While animal AO is capable of producing superoxide as well as hydrogen peroxide by transferring electrons to molecular oxygen [97] plant AO was shown to generate hydrogen peroxide only [73].

In most animals AO is encoded by one gene, thus leading to one homodimeric enzyme being most abundant in the liver and the lung [98]. In contrast to XDH much less is known about the physiological function of animal AO as even its native substrates are yet unravelled. AO is capable of converting retinaldehyde into retinoic acid ([99] [Terao, 2001 #711]) [100] which is the active metabolite of vitamin A, a known morphogen [101] and a key regulator of many tissues and cell types in the adult animal. Thus, animal AO may be of relevance for development and may control the homeostasis of certain types of tissues. The enzyme has also been implicated in the hepatotoxicity of ethanol in humans and other mammals by oxidizing the toxic metabolite acetaldehyde into acetic acid [102]. Besides AO other AO-homologs were found in mice and rats, represented by AOH1, AOH2 and AOH3 [99,103]. The physiological functions of these AO-homologs are even less clear and remain to be shown.

In contrast to AO from animals the physiological importance of the plant counterpart is much better understood. The *A. thaliana* genome harbors four AO genes, *AAO1–AAO4*, whose products form homodimers as well as heterodimers thus leading to altered substrate specificities of the respective isoenzymes. In 6-day-old seedlings, three AO isoenzymes can be detected capable of producing indole-3-acetic acid and 1-naphthyl acetic acid [104], respectively, both belonging to the auxin family of plant hormones. During plant development the isoenzyme composition changes, thereby generating AOδ [105] which acts best with abscisic aldehyde as substrate. Abscisic aldehyde is the native precursor of the plant hormone abscisic acid which is essential for many developmental processes as well as for a variety of abiotic and biotic stress responses [106–108]. Hence, AO enzymes in plants are essential for many physiological processes that require the involvement of the hormones abscisic acid and/or auxines.

14. Sulfite oxidase

SO (EC 1.8.3.1), the name-giving enzyme for all members of the SO-family of Mo-enzymes, catalyzes the oxidation from sulfite to sulfate, the final step in the degradation of sulfur-containing amino acids. While the animal enzyme basically consists of an N-terminal iron-heme containing cytochrome b_5 domain and a C-terminal domain responsible for Moco binding and dimerization [12] (Fig. 2A), its counterpart from plants is lacking the cytochrome b_5 domain [109,110]. Thus, plant SO is the simplest Mo-enzyme found in eukaryotes with its intramolecular redox center only consisting of Moco. Oxidation of sulfite is a two-electron transfer reaction in which the electrons from sulfite reduce the Mo-center from Mo^{VI} to Mo^{IV}. In case of the plant enzyme the electrons are subsequently transferred to molecular oxygen with simultaneous formation of hydrogen peroxide [111]. In the animal enzyme, they are transferred to the heme Fe^{III} of the cytochrome b_5 domain and finally from Fe^{II} to the external electron acceptor cytochrome *c*.

SO from animals is located in the intermembrane space of mitochondria and its activity is indispensable to life as a lack of SO activity is connected to severe neurological abnormalities and early death [17]. In contrast, plant SO is a peroxisomal protein [112] and its physiological role is as yet not absolutely clear. As sulfite is a strong nucleophile that can react with a wide variety of cellular components it might well be that SO is required for removing excess sulfite from the cell, which accumulates either upon decomposition of methionine and cysteine or which is derived from high concentrations of SO_2 gas in the atmosphere [113,114]. Recent results indicate that the latter point indeed is of importance as SO-knockout plants from *A. thaliana* were found to be more susceptible to high concentrations of SO_2 than wildtype plants (R. Hänsch, R.R. Mendel, unpublished data). However, as the enzyme is capable of producing hydrogen peroxide its function might also be connected to ROS metabolism.

15. Nitrate reductase

NR (EC 1.6.6.1) which does not occur in animals 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. Like AO and XDH it also consists of three distinct domains, however, of different order and composition (Fig. 2A). The N-terminal domain of an NR monomer is specified by a bound Moco and is followed by a heme-binding cytochrome b_5 domain and a C-terminal FAD-binding domain, whereby two such monomers form the active homodimeric enzyme. The domains are separated by solvent-exposed linker regions, called hinge I and hinge II. In plants hinge I, the linker between the cytochrome b_5 domain and the Moco/dimerization domain, contains a conserved serine residue which mediates contact with a 14-3-3 protein when phosphorylated, subsequently leading to inhibition of enzyme activity [115]. An N-terminal extension preceding the Moco

domain was shown to be important for the post-transcriptional regulation of NR by light [116], but is not required for enzyme activity.

In contrast to the reactions as catalyzed by AO, XDH and SO the process of nitrate reduction consumes rather than produces electrons deriving either from NADH or NADPH. While NADH-specific forms of NR are most abundant in higher plants and algae, NADPH-specific forms were exclusively found in fungi. However, bispecific forms, capable of using NADH as well as NADPH, have been found in all these organisms, mostly in fungi [117]. The intramolecular electron transfer starts with the reductive half-reaction, characterized by the reduction of FAD by NAD(P)H. The electrons are then transferred via the cytochrome b_5 domain into the oxidative half-reaction at the Mo-center, where nitrate is reduced to nitrite by concomitant release of hydroxide [118]. While in terms of nitrogen assimilation nitrite is further reduced in the chloroplasts to ammonium by nitrite reductase, it can also be reduced to nitric oxide (NO) by NR itself [119,120]. It was shown that NR, purified and in crude extracts, produces NO at saturating NADH and nitrite concentrations at about 1% of its nitrate reduction capacity in vitro [121]. However, 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. Thus, the signalling molecule NO generated by NR might well contribute to plant growth development [122], protection against cytotoxicity of ROS [122] and accumulation of phytoalexin [123], but also to plant pathogen resistance [124] by increasing cGMP and salicylic acid levels.

16. Post-translational sulfuration of molybdenum-hydroxylases

Mo-enzymes in plants and humans can be 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. These enzymes need the addition of a terminal inorganic sulfur to the Mo-center in order to gain enzymatic activity. This sulfur ligand does not originate from the apoprotein nor does it come from the Moco moiety [125,126]. It was demonstrated that in vitro this sulfur can be spontaneously lost or can be removed from XDH/AO by cyanide treatment generating an inactive enzyme [127]. The reaction, however, is reversible and the enzyme can be reactivated by sulfide-treatment under reducing conditions. In vivo, this terminal sulfur has to be added by a separate enzymatic reaction. Bittner et al. [128] identified the protein ABA3 from *A. thaliana* as Moco-sulfurase catalyzing the insertion of the cyanolyzable sulfur into the active center of XDH and AO, thus leading to activation of these enzymes.

ABA3 is a two domain protein acting as a homodimer. Its N-terminus shares significant homologies to bacterial cysteine desulfurases, thereby being more similar to SufS than to NifS or IscS. In a pyridoxal phosphate-dependent manner, the N-terminal domain of ABA3 decomposes L-cysteine to yield alanine and elemental sulfur, the latter being bound as a persulfide to a highly conserved cysteine residue of ABA3 (Fig. 3) [129]. As the C-terminal domain of ABA3 recently was found to efficiently bind sulfurated Moco (S. Wollers, F. Bittner, unpublished data), it appears likely that the persulfide sulfur is transferred from the N-terminal domain to Moco bound at the C-terminal domain prior to activation of the respective target enzyme. It is still unclear whether ABA3 finally activates its target enzymes by transferring only the sulfur, which is bound to the Moco of the C-terminal domain, or whether it transfers the entire sulfurated Moco of the C-terminus in order to activate XDH and AO. In the first case the C-terminus thus would act as a scaffold for the assembly of a Mo-S center of which only the sulfur is subsequently passed to the Moco of the target enzymes. In the second case the reaction catalyzed by ABA3 would be an exchange reaction where non-sulfurated Moco of the inactive target enzymes is replaced by sulfurated Moco from the C-terminal domain of ABA3.

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 [130], thereby being consistent with the conditions required for induction of AO and XDH [73,83,106].

17. Deficiencies of molybdenum-enzymes and of molybdenum cofactor sulfurase

While Moco deficiency results in the pleiotropic loss or reduction of all Mo-enzyme activities isolated Mo-enzyme deficiency is characterized by the loss or reduction of the activity of only one particular Mo-enzyme.

In humans the most severe phenotype is observed upon isolated SO deficiency which is a very rare disease, mostly accompanied by death at early childhood [16,17]. Typical symptoms of SO deficiency are mental retardation, attenuated growth of brain and dislocated ocular lenses [17] and can be primarily ascribed to either elevated levels of sulfite or to reduced levels of sulfate, or to a combination of both: high amounts of sulfite are toxic for the organism, in particular for the brain, while sulfate is required for sphingolipid formation which constitute major components of the myelin sheath [131]. SO deficiency is clinically indistinguishable from Moco deficiency as the effects of XDH and AO in Moco patients are negligible. In contrast to human SO deficiency the lack of SO activity in plants is not related to an obvious phenotype. Only when SO mutants are exposed to high concentrations of SO_2 gas a reduced resistance towards this particular stress can be observed (R. Hänsch, R.R. Mendel, unpublished data).

In comparison to isolated SO deficiency in humans many more patients were found with inherited isolated XDH deficiency, referred to as xanthinuria type I. This autosomal-

recessive disorder may lead to kidney stone formation, urinary tract disorders, acute renal failure and muscle diseases [132], but until recently no other physiological symptoms have been found to be associated with reduced XDH activity. However, heterozygous XDH knockout-mice were found to be unable to maintain lactation due to collapsed mammary epithelium while mice, homozygous for the XDH deficiency, died at latest 6 weeks after birth [86]. With respect to this particular phenotype the importance of the structural function of XDH rather than the enzymatic one is discussed. From this observations the authors conclude human females suffering from xanthinuria may be potential candidates for lactation problems. It is noteworthy that increased XDH activity, referred to as hyperuricemia, is associated with elevated levels of uric acid in the serum which may lead to formation of urate crystals in the joint regions, thus leading to gout. As allopurinol is a well known inhibitor of XDH enzymes it is commonly used for therapy of hyperuricemia and gout patients.

Contrary to the isolated deficiencies of SO and XDH no case of isolated AO deficiency is reported in humans or other vertebrates until now. In plants severe phenotypes have been observed when those AO isoforms were mutated that are responsible for the production of abscisic acid. The AO mutants *aao3* from *A. thaliana* [133] and *sitiens* from tomato [134], both characterized by dramatically reduced levels of the phytohormone abscisic acid, suffer from excessive loss of water and are unable to respond to environmental stresses, and thus are unable to survive in nature. However, mutations in any of the other *A. thaliana* AO genes have not been reported to be associated with phenotypes.

As NR is specific for autotrophic organisms isolated NR deficiency in eukaryotes has been reported only for plants [135–137], fungi [138] and algae [139]. Such mutants have lost the ability to grow on nitrate medium as the sole nitrogen source and thus are dependent on alternative nitrogen sources such as ammonium. However, as some organisms, e.g., *A. thaliana*, express a second NR gene, the deficiency in one can be compensated by the other [140].

Moco-sulfurase deficiency in vertebrates and plants is characterized by the simultaneous loss of XDH and AO activities while the activities of SO, and in plants also NR, are preserved. The disease in mammals, referred to as xanthinuria type II, can be differentiated from xanthinuria type I by means of the allopurinol loading test [141]. As allopurinol is converted to oxypurinol by both, XDH and AO, upon application of allopurinol oxypurinol is detected in urine of patients with classical xanthinuria type I, but not in urine of those with xanthinuria type II. However, no other difference can be found between type II and type I patients, indicating that the loss of XDH activity is the primary cause of the type II phenotype [142]. In plants Moco-sulfurase deficiency was found to be basically ascribed to the reduction of abscisic acid levels due to the lack of AO activities [130,143]. The phenotype is even worse in comparison to mutants with a single mutation in any of the AO or XDH genes, as all the important processes controlled by AO and XDH enzymes are disturbed in Moco-sulfurase mutants, thus presenting an overlapping phenotype.

18. Crosstalk between molybdenum and iron metabolism

In eukaryotes, the Moco biosynthesis pathway is intimately linked to the homeostasis of other metals: (1) copper is essential for protecting the highly reactive dithiolene group of MPT before molybdenum is inserted for Moco completion (Figs. 2 and 3). (2) The first step of Moco-biosynthesis, i.e., the conversion of GTP to precursor Z, requires the availability of iron as Cnx2 in plants and Mocs1A in mammals are iron–sulfur [Fe–S] cluster-binding proteins (Fig. 3). Two [Fe–S] clusters of the [4Fe–4S] type are essentially involved in the *S*-adenosylmethionine-dependent mechanism of precursor Z formation [27] and have to be preassembled in the mitochondria, where mitochondrial as well as extramitochondrial [Fe–S] clusters originate from [144]. (3) Iron in the form of [2Fe–2S] clusters is required by XDH and AO, and in the form of iron-heme by NR and animal SO.

The importance of the crosslink between iron and Mo was found recently by investigating the *sta1* mutant of *A. thaliana*. It was shown that the ABC transporter Sta1 in the membrane of mitochondria is a homolog of Abc7 and MTABC3 from humans and mediates the export of compounds required for cytosolic [Fe–S] clusters from mitochondria into the cytosol [145]. The *sta1* mutant was shown to have wildtype amounts of total iron, but to accumulate free (non-heme and non-protein) iron in the mitochondria as iron for extramitochondrial [Fe–S] proteins is not exported. In this mutant also Moco biosynthesis has been found to be affected because precursor Z levels were elevated while MPT amounts were reduced (J. Teschner, F. Bittner, unpublished data). A possible explanation could be that besides the export of [Fe–S] clusters Sta1 also mediates export of a sulfur-containing compound which is the sulfur substrate for the second step of cytosolic Moco synthesis (conversion of precursor Z into MPT by formation of the dithiolene group). Another possible explanation might well be that in addition to [Fe–S] clusters Sta1 also mediates the export of precursor Z, thus requiring the proteins of the first step of Moco biosynthesis, Cnx2 and Cnx3, to be located in mitochondria rather than in the cytosol.

To this end it is not clear what other compounds besides [Fe–S] constituents are exported by Sta1, but it is obvious that Sta1 bridges iron/[Fe–S]- and Mo-metabolism as it is essential for exporting a Moco biosynthesis-related molecule. This is supported by the Mo-enzyme activities of the *sta1* mutant: NR activities are reduced in the same manner as MPT amounts while the activities of XDH and AO are nearly completely abolished, indicating that NR is affected solely by the reduction of Moco amounts while XDH and AO are additionally affected by the non-availability of cytosolic [Fe–S] clusters, which they require as prosthetic group.

As each of the three basic steps in Moco biosynthesis depends on a different metal (step 1 = Fe, step 2 = Cu, step 3 = Mo), a possibility is provided for the cell to sense supply and

demand of these metals as well as of substrates and products of Moco biosynthesis.

19. Outlook

Our understanding of the biological role and the function of Mo is progressing rapidly. Regardless of many breaking results, there is still a large number of unresolved questions that need to be answered. Now that most of the relevant genes are cloned and the basic function of the proteins encoded are known, research concentrates both on the detailed enzymology of Moco biosynthesis/allocation and on studying regulation and structure–function relationships of Mo-enzymes. How is the molybdate transporter organized in detail? The chemistry underlying precursor Z formation is still enigmatic, and proposed theories have to be proven. And, given the great medical relevance of precursor Z for the treatment of Moco-deficient patients, can it be synthesized chemically? How is the multienzyme complex for Moco biosynthesis organized? What can MPT synthesis mechanism tell us about the state of copper bound to MPT, and what is the role of copper in Moco synthesis in general? Is there any metabolic link between Mo and copper metabolism?

What is the mechanism of Moco insertion into apo-enzymes? How is Moco biosynthesis regulated to meet the changing demands of the cell for Moco?—The coming years will bring insight into the integration and (perhaps unexpected) regulatory connections of Moco-biosynthesis and Mo-enzymes within the metabolic and physiological network of the cell.

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