The Molybdenum cofactor
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The transition element molybdenum (Mo) needs to be complexed by a special cofactor in order to gain catalytic activity. Mo is bound to a unique pterin, thus forming the molybdenum cofactor Moco, which in different variants is the active compound at the catalytic site of all Mo-containing enzymes in nature, except bacterial Mo-nitrogenase. The biosynthesis of Moco involves the complex interaction of six proteins and is a process of four steps, which also requires iron, ATP and copper. After its synthesis, Moco is distributed involving Moco-binding proteins. A deficiency in the biosynthesis of Moco has lethal consequences for the respective organisms.

The transition element molybdenum (Mo) is an essential micronutrient for microorganisms, plants and animals (1). Surprisingly, Mo itself is catalytically inactive in biological systems until it is complexed by a special scaffold (2). One type of scaffold is the ubiquitous pterin-based Mo-cofactor (Moco) that in different variants forms part of the active centers of all Mo-enzymes in living organisms, except one enzyme. This exception is bacterial nitrogenase which harbors the other type of cofactor, namely the iron-sulfur cluster based iron-Mo-cofactor which is found only once in nature (for details compare the review of Ribbe in this issue). Mo belongs to the group of trace elements, i.e. the organism needs it only in minute amounts, however, unavailability of Mo is lethal for the organism. Mo is very abundant in the oceans in the form of the molybdate anion (3). In soils, the molybdate anion is the only form of Mo that is available for bacteria, plants and fungi. Hitherto more than 50 enzymes are known to be Mo-dependent. The vast majority of them are found in bacteria while in eukaryotes only seven have been identified (4, 5). Somewhat surprising not all organisms need Mo. The commonly used eukaryotic model organism yeast plays no role in Mo research as Saccharomyces cerevisiae neither contains Mo-enzymes nor the Moco biosynthesis pathway. Also S. pombe does not use Mo while Pichia pastoris needs Mo.

Genome-wide database analyses revealed a significant number of bacteria and unicellular eukaryotes that do not need Mo while all multicellular eukaryotes are dependent on Mo (6). In addition, mainly anaerobic archaea and some bacteria are Mo-independent but they require tungsten (W) for their growth (7). In the periodic table of elements, W lies directly below Mo and features chemical properties similar to Mo.

Mo metabolism is tightly connected to iron-sulfur (Fe-S) cluster synthesis in that some of the Mo-enzymes and Moco biosynthesis itself depend on Fe-S enzymes and on a mitochondrial transporter that is known to be crucial for the maturation of cytosolic Fe-S proteins (for details compare the review of Lill in this issue). Moreover, Moco biosynthesis has recruited mechanisms previously known from Fe-S cluster synthesis, which involve the mobilization of sulfide for the formation of a Mo-sulfur center for specific Mo-enzymes which will be touched upon below.

What is the Molybdenum Cofactor?

Mo has a versatile redox-chemistry that is used by the enzymes to catalyze diverse redox reactions. Mo-enzymes generally catalyze the transfer of an oxygen atom, ultimately derived from or incorporated into water, to or from a substrate (5). Each reaction, either reduction or oxidation, involves the transfer of two electrons, thereby causing a change of the oxidation state of the Mo atom in the substrate-binding site from IV to VI or vice versa. Most remarkably, it turned out that the metal is not directly attached to the catalytic site, rather the Mo atom is complexed within a specific, low-molecular scaffold in order to fulfil its catalytic function. This compound is a unique tricyclic pterin called molybdopterin or metal-containing pterin (MPT) (Fig. 1), the latter reflecting the fact that in bacteria not only Mo but also W can be coordinated by this pterin scaffold which seems to be the rule in archaea that prefer W instead of Mo. As the result of Mo coordination by MPT, Moco is formed (2). The chemical nature of Moco was elucidated by the work of J.L.
Johnson and K.V. Rajagopalan (8) (Fig. 1). As Moco turned out to be very labile and sensitive to air-oxidation, its stable oxidation products were used to uncover its pterin nature. Moco, however, proved to be a unique pterin derivative because it possessed a four-carbon side chain as C6 substituent that coordinated Mo via an enedithiolate group located within the four-carbon side chain of the cofactor (9). Finally, crystal structures of Mo-enzymes confirmed this core structure and showed the existence of a third pyranon ring between the OH-group at C3′ of the side chain and the pterin C7 atom (10). Once the pyranon ring is closed, a fully reduced hydrogenated pterin is formed. In prokaryotes, variants of Moco occur: (i) Moco can bind a nucleotide (GMP or CMP) to its phosphate thus forming a dinucleotide cofactor, and (ii) Mo can be coordinated by two pterin or two dinucleotide equivalents thus forming bis-forms of Moco. In this review I will focus on higher organisms (eukaryotes). Bacterial Moco variants were recently reviewed in detail (11).

What could be the task of the pterin moiety of Moco? The fusion of a pterin with a pyranon ring as identified for Moco and is direct precursor, the metal free MPT, is unique in nature and may have been evolved in order to position the catalytic metal correctly within the active center of a given Mo-enzyme. Another possible role of the pterin moiety could be control of the redox behavior of the Mo atom. In addition, the pterin might also participate in the electron transfer to or from Mo via the delocalized electrons within the pterin (5). 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 cognate substrates (12, 13). During its life time, the Mo-enzyme does not liberate Moco. In vitro however, Moco can be removed from its protein environment whereafter it easily loses the Mo atom and becomes rapidly oxidized, resulting in an irreversible loss of function. The demolybdo-forms of Mo-enzymes are catalytically inactive. To this end there are no indications for a Moco recycling mechanism in the cell.

Molybdenum Uptake into Cells

Mo is taken up in the form of its oxyanion molybdate. In the presence of competing anions it requires specific uptake systems that were studied in detail in bacteria where high-affinity ABC-type transporters are described requiring ATP-hydrolysis for operation (7). In higher organisms, only recently first molybdate-transporting proteins have been identified in algae and plants (14-16). Among the large sulfate carrier superfamily two proteins, Mot1 and Mot2, were shown to transport molybdate with ultra-high affinity (nanomolar kM value) across cellular membranes. Surprisingly, none of them was found to reside in the plasmamembrane. Contradictory reports localized Mot1 to the endomembrane system (14) and to the mitochondrial envelope (16), respectively. The latter however is questionable as the insertion of Mo into the Moco-backbone takes place in the cytosol. For Mot2, GFP fusion proteins have shown that the protein localizes to the vacuolar membrane (17). Molybdate quantification in isolated vacuoles demonstrated that this organelle serves as an important molybdate storage compartment in Arabidopsis thaliana cells where Mot2 was shown to be required for vacuolar molybdate export into the cytosol. The major question remains how molybdate is entering the cell. The answer might come from recent results from the alga C. reinhardtii where another molybdate transporter has been identified that unlike Mot1 and Mot2 is not exclusively found in algae and higher plants but also occurs in humans (18). Although still not localized it is likely that this transporter serves as the general molybdate importer for the cell. Further, it appears that in addition to the specific high-affinity uptake system molybdate may also enter the cell non-specifically through the sulfate and the phosphate uptake system. Molybdate uptake through a sulfate transporter has recently been described, thus supporting this assumption (19).

Molybdenum Cofactor Biosynthesis

Early genetic work demonstrated that mutations in the genes for Moco biosynthesis result in the pleiotropic loss of all Mo-dependent cellular processes. Analysis of Moco-deficient mutants in a given organism ranging from bacteria to plants and humans resulted in the identification of several gene loci being involved in Moco biosynthesis (2, 11). Along with the conserved structure of Moco, these findings provided a basis to propose an evolutionary old multi-step biosynthetic pathway (20). The first model for Moco biosynthesis was presented by Rajagopalan and coworkers for the bacterium E. coli (21). Later studies of Moco biosynthesis...
uncovered a more complex picture of this pathway in higher organisms where molecular, biochemical and genetic analyses of Moco mutants were most advanced in plants (22). These results formed the basis to decipher Moco biosynthesis also in humans and it turned out that the pathways of Moco biosynthesis showed a high degree of similarity in both organisms (2).

In all higher organisms studied so far, Moco is synthesized by a conserved biosynthetic pathway that can be divided into four steps, according to the biosynthetic intermediates cyclic pyranopterin monophosphate (cPMP, previously also known as precursor Z), MPT, adenlylated MPT (MPT-AMP), and Moco, respectively (Fig. 2). Among eukaryotes, always six proteins catalyzing Moco biosynthesis have been identified in plants (23), fungi (24) and humans (25–27). 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. Different nomenclatures were introduced for genes and gene products involved in Moco formation. Genes and the encoded proteins were named in plants according to the \textit{cnx} nomenclature (cofactor for nitrate reductase and xanthine dehydrogenase). For human Moco synthetic genes, a different MOCS (molybdenum cofactor synthesis) nomenclature was introduced, and the names for both the plant and human proteins are given in Fig. 2. A third nomenclature is used among bacteria. For comparison, Fig. 2 also shows the names of the corresponding bacterial proteins. In the following we will briefly characterize the individual steps of Moco biosynthesis.

**Biosynthesis Step 1: Conversion of GTP to cPMP**

Like the biosynthesis of other pteridines, Moco synthesis starts with guanosine 5'-triphosphate (5'-GTP), which is converted by a complex reaction sequence into cPMP (Fig. 2). Differently from the other pteridine pathways (producing three-carbon side chains), MPT is unique in having a four-carbon side chain. cPMP is the most stable intermediate of Moco biosynthesis with an estimated half life time of several hours at a low pH (28). Therefore it was possible to solve its structure (\textit{E. coli}) using \textit{'H}-NMR, while structural elucidation of MPT, MPT-AMP and Moco required crystallization of protein ligand complexes (29). cPMP already possesses a fully reduced tricyclic tetrahydro-pyranopterin structure and is predominantly hydrated at the C1' position resulting in a geminal diol (30). GTP labeling studies and \textit{'H}-NMR demonstrated that each carbon atom of the ribose and of the guanine ring are incorporated into cPMP. The underlying mechanism involves a complex radical-based rearrangement reaction in which the C8 atom of the purine is inserted between the 2' and 3' ribose carbon atoms, thus forming the new C1' position in the four-carbon side chain of the pterin (28, 31).

cPMP is first intermediate of Moco biosynthesis. It is still sulfur-free but it has already the tricyclic pyranopterin structure similar to the mature cofactor. In all organisms, the conversion of GTP to cPMP is catalyzed by two proteins, one of them (Cnx2 in plants, MOCS1A in humans) belongs to the superfamily of S-adenosylmethionine(SAM)-dependent radical enzymes (32). Members of this protein family catalyze the formation of protein and/or substrate radicals by reductive cleavage of SAM involving a [4Fe-4S] cluster. MOCS1A is a protein containing two oxygen-sensitive Fe-S clusters each coordinated by only three cysteine residues (32). For its bacterial homolog (MoaA-protein in \textit{E. coli} and in \textit{S. aureus}, respectively) the complex reaction mechanism has been deciphered in detail (33, 34). As the plant gene \textit{cnx2} (35) and the human gene \textit{mocs1A} (26) are able to functionally complement their bacterial counterpart one can assume that the reaction mechanism is likely to occur similarly in eukaryotes. The N-terminal [4Fe-4S] cluster binds SAM and carries out the reductive cleavage of SAM to generate the 5'-deoxyadenosyl radical, which subsequently initiates the transformation of 5'-GTP bound through the C-terminal [4Fe-4S] cluster. The function of the second protein involved in catalyzing step 1 (i.e. Cnx3 in plants and MOCS1B in humans) is as yet unknown but it is believed that it participates in pyrophosphate release upon the rearrangement reaction.

**Export of cPMP from Mitochondria, a Link between Moco and Fe-S Cluster Synthesis**

In eukaryotes, the two proteins involved in step 1 of Moco biosynthesis carry N-terminal extensions which predict a mitochondrial localization of these proteins. Subfractionation of mitochondria demonstrated that Cnx2 and Cnx3 reside in the matrix where 5'-GTP as substrate for cPMP synthesis is available and where Fe-S
clusters as the essential prosthetic group for Cnx2 are synthesized (36). In contrast to the first step, all subsequent steps of Moco biosynthesis were demonstrated to be localized in the cytosol (37-39) and thus, after its synthesis in mitochondrial cPMP has to pass the mitochondrial membranes to enable its further processing to Moco (Fig. 3). Although cPMP is hydrophobic enough to pass biological membranes simply by diffusion, recent work on plants demonstrated that a specific transporter in the inner membrane of mitochondria is involved in the transport of cPMP into the cytosol (36). The respective transporter belongs to the ATP-binding cassette (ABC) transporter family and in plants is referred to as ATM3. Interestingly, together with yeast Atm1p and mammalian ABCb7 this transporter was originally identified to be essential for the maturation of extramitochondrial Fe-S proteins by transporting an as yet unknown compound generated during mitochondrial Fe-S cluster synthesis into the cytosol, where it serves as substrate for the cytosolic Fe-S assembly machinery (review of Lill in this issue). It turned out that a loss of ATM3 function is not only associated with a deficiency in extramitochondrial Fe-S proteins but also with the accumulation of cPMP within mitochondria, which has the consequence that the cytosol becomes short of cPMP thus leading to decreased Moco levels and Mo-enzyme activities in the cell (36). The precise role of ATM3 is however still unknown.

Biosynthesis Step 2: Synthesis of Molybdopterin

In the second step, sulfur is transferred to cPMP in order to generate MPT. This reaction is catalyzed by the enzyme MPT synthase, a heterotetrameric complex (Fig. 2) of two small (Cnx7 and MOCS2B, respectively) and two large (Cnx6 and MOCS2A, respectively) subunits that stoichiometrically converts cPMP into MPT. The sulfur is bound to the C-terminus of the small subunits 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 that was deciphered in detail in bacteria (11). Among all small subunits so far analyzed from diverse species, the C-terminal region is highly conserved which includes a terminal double glycine that is of functional importance for thioisocarboxylation (40, 41). E. coli MPT synthase was found to be an elongated protein complex where the thiocarboxylated C-termini of the small subunits are deeply inserted into the large subunits from to two clearly separated active sites (42). Obviously the two sulfur atoms are not simultaneously transferred to cPMP, rather the sulfurs become sequentially inserted starting with C2’ of cPMP with the consequence that a monosulfurated reaction intermediate will occur (43). Whether the intermediate will be transferred within the MPT synthase to the other active site or whether the enzyme dissociates to host another sulfurlated small subunit remains to be seen. Again, like in step 1, also the reaction mechanism of MPT synthase is conserved between bacteria and higher organisms as, at least for the large subunits, proteins can be exchanged between organisms (41).

After MPT synthase has transferred the two sulfurs to cPMP, it has to be re-sulfurated by the enzyme MPT-synthase sulfurase (Cnx5 and MOCS3, respectively; Fig. 2) in order to re-activate the enzyme for the next reaction cycle of cPMP conversion. This re-sulfuration involves an adenylation of MPT synthase small subunit followed by the sulfur transfer. At this stage the sulfur transfer reaction in higher organisms appears to involve different protein components as the eukaryotic genes cannot complement their bacterial counterparts. MPT-synthase sulfurase is a two-domain protein consisting of a N-terminal adenylation domain (homologous to E. coli MoeB) and a C-terminal rhodanese-like domain (RLD) where the sulfur is bound to a conserved cysteine in form of a persulfide (38) (44). In analogy to the bacterial mechanism, this enzyme is supposed to activate the small subunit of MPT-synthase by adenylation followed by sulfur transfer (coming from the rhodanese-like domain), thus forming the thiocarboxylate at the C-terminus of the small subunit (2). Therefore MPT-synthase sulfurase can be seen as a multi-functional protein combining two subsequent reactions carried out by two domains fused to each other (Fig. 2), thus representing a good example of product-substrate channeling. In humans, cysteine desulfurase Nfs1 is a likely candidate to function as sulfur donor for the MOCS3-catalyzed resulfuration step (45).

Biosynthesis Step 3: Mo insertion starts with Adenylation of Molybdopterin

After synthesis of the MPT moiety, the chemical backbone is built to bind and coordinate the Mo atom (Fig. 2). Therefore, in
the third step molybdate is transferred to MPT in order to form Moco, thus linking the molybdate uptake system to the MPT pathway, which however, is not a spontaneous process but is catalyzed by a Mo-insertase. Mutants defective in this step accumulate MPT but can be partially rescued by growing them on high-molybdate (1 – 10 mM) media, which is used as an assay tool to identify Mo-insertase mutants (46).

Physiological molybdate concentrations, however, are not sufficient to achieve any non-catalyzed Mo ligation by MPT. In bacteria, this step is catalyzed by two separately expressed proteins (MogA and MoeA) while during evolution to higher organisms these two proteins were fused to a single two-domain Mo-insertase (Cnx1 in plants and Gephyrin in mammals). The two domains of Mo-insertase are named G-domain (homologous to MogA) and E-domain (homologous to MoeA) (Fig. 2), and work with the Mo-insertase Cnx1 from the model plant Arabidopsis thaliana assigned different mechanistic functions to each of these domains (47, 48).

The metal insertion reaction can be subdivided into two separate steps. Structural studies revealed that in order to coordinate Mo, MPT at first has to be activated by adenylation. This is carried out be the Cnx1 G-domain in a Mg$^{2+}$- and ATP-dependent manner, thus generating MPT-AMP. The finding that MPT-AMP represents a general reaction intermediate in Moco biosynthesis was further extended by recent studies in E. coli (11). MPT-AMP serves as substrate for the subsequent Mo insertion reaction which is carried out be the E-domain of Cnx1.

**Biosynthesis Step 4: Mo insertion into Molybdopterin**

In the final step, MPT-AMP is transferred from Cnx1G to the Cnx1 E-domain that cleaves the adenylate from MPT and catalyzes the insertion of molybdate into the dithiolene group of MPT, thus yielding physiologically active Moco (Fig. 2). The MPT adenylate is hydrolyzed in a Mg$^{2+}$-dependent and molybdate-dependent way, and adenylated molybdate might occur as hypothetical reaction intermediate (47, 48). Moco formed by Cnx1E most probably carries two oxo ligands and one OH-group in a deprotonated form (48). There is no experimental evidence for a reduction of Mo at this stage.

The crystal structure of the Cnx1G revealed an unexpected finding, namely a copper bound to the MPT dithiolate sulfurs, whose nature was confirmed by anomalous scattering of the metal (29) (1). These structures show tetragonal coordination of Cu suggesting a type I Cu binding site for Cu$^{1+}$. Given the presence of Cu in MPT, the insertion of Mo into the MPT dithiolene group can be characterized as metal exchange reaction, with Cu presumably serving as suitable leaving group. It is also possible that Cu is protecting the MPT dithiolate from oxidation. The origin of this Cu is still unclear but it is reasonable to assume that it binds to the ededithiolate group just after the latter has been formed, i.e. at the end of step 2 of Moco biosynthesis. Since in vivo Cu occurs exclusively protein-bound it is likely that both, Cu binding to MPT and its exchange for Mo, depend on yet unidentified cytoplasmic chaperones involved in cellular Cu metabolism.

**Product-Substrate Channeling in Moco Biosynthesis**

Bacteria catalyze step 3 and step 4 of Moco biosynthesis by separate proteins (MogA and MoeA, respectively) while higher organisms combined these two consecutive steps into a single protein (plant Cnx1, human Gephyrin) with two domains. Both domains were fused at least two times during evolution resulting in two-domain proteins with different orientations of the G and E domains: plants have the E-domain on the N-terminus of the protein, in mammals and fungi the G domain is on the N-terminus (2, 27).

These evolutionary distinct events point to a high pressure as well as functional benefit of having the adenylation function and the metal insertion function coupled into one protein where the fragile intermediate MPT-AMP is channeled from the G-domain to the E-domain (49).

Clearly, facilitated product-substrate flow seems to be a general characteristic of the Moco biosynthesis cascade. Indeed it was recently found that Cnx5, Cn6/7 an Cnx1 (catalyzing steps 2 through 4) undergo tight protein-protein interaction in the cytosol of living plant cells (39) thus supporting the idea of channeling the fragile intermediate MPT-AMP is channeled from the G-domain to the E-domain (49).

**Storage and Transfer of Moco**

Moco is extremely sensitive to oxidation (21) and therefore is assumed to occur permanently protein-bound in the cell. Also the fast flow of Moco to its target enzymes is an essential prerequisite to reduce the threat of Moco oxidation.
degradation. Both preconditions may be met by Moco binding proteins (MoBP) ensuring Moco binding as well as its directed transfer to cognate target enzymes. Thus, a pool of insertion-competent Moco may be stored and provided on demand. Among eukaryotes, a first MoBP named Moco carrier protein (MCP) was identified in the green algae Chlamydomonas reinhardtii (50). The protein is able to bind and protect Moco against oxidation and the atomic structure showed that it forms a homotetramer capable of holding four molecules of Moco (51). Without any denaturing procedure, subsequent transfer of Moco from the carrier protein to apoenzyme reductase (NR) from Neurospora crassa was possible. It is however unknown whether MCP is also able to donate Moco to Mo-enzymes other than NR. Preliminary data suggest that Mo is bound in a tri-oxo coordinated form in MCP. However, a complex structure of MCP with Moco is still missing.

In the higher plant Arabidopsis thaliana, a family of eight MCP-related proteins was identified that all can bind Moco (52). Their biochemical characterization showed reversible Moco binding properties, however with overall lower affinities. Therefore, these MoBPs are not good candidates to serve as Moco storage proteins. Rather they seem to be involved in the cellular distribution of Moco since they were found to undergo protein-protein interactions both with the Moco-donor protein Cnx1 and the Moco-user protein NR (Fig. 3). This observation does not exclude a direct transfer of Moco from the donor protein Cnx1 to the Mo-enzyme, which has been shown in vitro.

Insertion of Moco into Mo-enzymes is still not understood. All crystal structures of Mo-enzymes demonstrated that Moco is deeply buried within the holo-enzymes (12). Hence it follows that Moco needs to be incorporated prior to or during completion of folding and dimerization of the apoprotein monomers. 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. Nearly each bacterial Mo-enzyme has a private chaperone available (4). However, so far no eukaryotic Moco chaperones have been identified.

Final sulfuration of Moco

Two different Mo-enzyme families are known in eukaryotes (5): (i) The sulfite oxidase family to which also NR and the mitochondrial amidoxime reducing component mARC belong, and the (ii) xanthine oxidase family to which also aldehyde oxidase belongs. In bacteria, a third class of Mo-enzymes is known where two MPT equivalents coordinate one Mo atom (4). It is assumed that the rare eukaryotic Mo-enzymes pyridoxal oxidase (53) and nicotinate hydroxylase (54) represent specific isoforms of aldehyde oxidase. In contrast to the sulfite oxidase family, the members of the xanthine oxidase family require a final step of maturation prior to or after insertion of Moco. In addition to the dithiolene sulfurs of the pterin moiety and two oxo-groups, the Mo-atom of the Moco needs the addition of a terminal inorganic sulfur in order to provide enzymatic activity to these enzymes (55). This final step is catalyzed by the Moco sulfurate protein (ABA3 in plants, HMCS in humans) (Fig. 3). ABA3 is a homodimeric two-domain protein (56) with its N-terminal domain sharing structural and functional homologies to bacterial cysteine desulfurases, thereby being more similar to SuS 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 (57), the latter being bound as a persulfide to a highly conserved cysteine residue of ABA3. The C-terminal domain of ABA3 shares a significant degree of similarity to the newly discovered mARC proteins and was shown to bind sulfurated Moco, which receives the terminal sulfur via an intramolecular persulfide relay from the N-terminal domain (58, 59). It is likely that subsequent to Moco-sulfuration, ABA3 exchanges non-sulfurated for sulfurated Moco thus activating its target Mo-enzyme.

Moco Deficiency and Therapy in Humans

Human Moco deficiency is a rare recessive hereditary disorder, which ultimately results in the death of affected patients. Lack of Moco biosynthesis results in the pleiotropic loss of all Mo-dependent enzyme activities (2). Symptoms develop shortly after birth, when the babies’ metabolism starts to operate and toxic metabolites (mainly sulfite which is formed upon degradation of sulfur-containing amino acids) accumulate within the body (60). Two thirds of the patients turned out to have a defect in step 1 of Moco biosynthesis (= formation of cPMP). First human exposure of cPMP treatment has been reported recently (61) where a patient has been diagnosed on day 6 of life and experimental
treatment was started on day 36 of life. Within days, all biomarkers returned to almost normal readings and stayed constant.

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**Abbreviations and Acronyms**
cPMP, cyclic pyranopterin monophosphate; Moco, molybdenum cofactor; MoBP, molybdenum cofactor binding protein; MPT, molybdopterin; NR, nitrate reductase;
REFERENCES


FIGURE LEGENDS

FIGURE 1: Moco in eukaryotic Mo-enzymes. There are two different Mo-enzyme families known in eukaryotes: In enzymes of the sulfite oxidase family, X is represented by a single-bonded sulfur provided by a cysteine residue of the respective protein while Y corresponds to a double-bonded oxygen. In enzymes of the xanthine oxidase family, X is represented by a double-bonded inorganic sulfur and Y by a hydroxyl group.

FIGURE 2: Biosynthesis of eukaryotic Moco. The biosynthesis pathway is divided into four steps, as given in italics on the right side. On the left side, the names for the proteins from plants (green), humans (red) and E. coli (black) catalyzing the respective steps, are given. For MPT and MPT-AMP, the ligands of the dithiolate sulfurs are indicated by an “R” as it is currently unknown at which step copper is bound to the dithiolate. In GTP, the C8 atom of the purine is labeled with a star. This carbon is inserted between the 2’ and 3’ ribose carbon atoms, thus forming the new C1’ position in the four-carbon side chain of the pterin (labeled with a star in cPMP). In step 2, the heterotetrameric MPT synthase complex converts cPMP into MPT. In this process, two sulfur atoms need to be transferred from the thiocarboxylated C-termini of the small subunits, which later form the dithiolene group of the MPT. Once having transferred their sulfur atoms, the small subunits need to be reloaded with sulfur, which is facilitated by the MPT synthase sulfurase. This enzyme consists of two domains with the N-terminal adenylation domain (AD) catalyzing the Mg-ATP-dependent adenylation at the C-terminal carboxy group of the small subunit of MPT synthase and the C-terminal rhodanese-like domain (RLD) being responsible for subsequent sulfur transfer. In step 3, the two-domain protein Mo-insertase catalyzes the Mg-ATP-dependent adenylation of MPT at its G-domain with subsequent transfer of MPT-AMP to its E-domain. In step 4 and occurring at the E-domain, MPT-AMP is deadenylated and the molybdate anion is incorporated to form the mature Moco.

FIGURE 3: Organization of biosynthesis and distribution of Moco in higher organisms (plants). Moco biosynthesis starts in the mitochondria. The enzymes Cnx2 and Cnx3 catalyze the S-adenosylmethionine(SAM)-dependent conversion of GTP to cPMP. Cnx2 requires Fe-S clusters provided by the mitochondrial Fe-S synthesis machinery, which also generates an as yet unknown compound that is exported by the ABC-type transporter ATM3 to allow synthesis of cytosolic Fe-S clusters. However, ATM3 is also involved in the transport of cPMP from mitochondria into the
cytosol where MPT-synthase adds two sulfur atoms and converts cPMP to MPT. The small subunit Cnx7 of MPT-synthase which holds the transfer-ready sulfur atom has received this sulfur from the enzyme MPT-synthase sulfurase Cnx5. AD denotes the adenylation domain of Cnx5 which is required for adenylation and activation of Cnx7 while the sulfur is mobilized by the rhodanese-like domain (RLD). It is assumed that copper (Cu) is inserted directly after dithiolene formation. The individual reactions of Mo-insertase Cnx1 and its products (Moco, pyrophosphate PPi, AMP, Cu) are indicated. Mature Moco can be either bound to a Moco-binding protein (MoBP), or directly to the Mo-enzymes or to the Moco-binding domain (MocoBD) of the Moco sulfurase ABA3. The Moco sulfurase generates a protein-bound persulfide, which is the source of the terminal sulfur ligand of Moco in xanthine oxidoreductase and aldehyde oxidase. Unlike Cnx2, the latter two enzymes depend on cytosolic Fe-S clusters.
Mendel: Figure 1