to each other, and define an electron-transfer pathway (Figure 1). Whereas the first Fe/S centre b is quite exposed to the solvent via its Cys-60, Fe/S centre a is buried approx. 15 Å below the molecular surface. The closest distance between the iron atoms of the two Fe/S centres is about 12 Å, and the molybdenum atom lies 15 Å away from the nearest iron atom of centre a. The molybdenum site is also buried but accessible to the protein surface through a deep tunnel as described.

The bound inhibitory propan-2-ol in the inner compartment of the substrate-binding tunnel is a model for the Michaelis complex of the reaction of Mop with aldehydes (RCH=O) (Figure 2). The reaction is proposed to proceed by transfer of the molybdenum-bound water molecule as OH−, after proton transfer to Glu-869, concertedly with hydride transfer to the sulphido group to generate: MoV, −O, −SH, −−−RCOO−. The dissociation of the carboxylic product may be facilitated by transient binding of Glu-869 to the molybdenum atom. The metal-bound water is then replenished from a chain of structural internal water molecules. A second alcohol-binding site, located in a wider part of the tunnel (Figure 1), may explain the strong substrate inhibition observed in Mop [5] as well as in xanthine oxidase [6]. This is also the putative binding site of large inhibitors of xanthine oxidase [7].

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**Biosynthesis and processing of the molybdenum cofactors**

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Structural studies in our laboratory on the cofactor associated with liver sulphite oxidase revealed the presence of a unique pterin component that has been termed molybdopterin (MPT; see Figure 1 for the proposed structure) [1,2] and subsequently led to the identification of several dinucleotide forms [3–6], including molybdopterin guanine dinucleotide (MGD, Figure 1), the component of the cofactor present in all *Escherichia coli* molybdoenzymes. The unusual features of the 6-alkyl side chain of the pterin, with four carbons, a terminal phosphate ester and the unique dithiolene group critical for metal ligation, suggested that the assembly of such a cofactor might involve heretofore undescribed enzymes and chemical pathways. Indeed studies on MPT biosynthesis have shown that the pathway involves several novel reactions as described below. Much of the progress in this area has been achieved through use of MPT mutants of *E. coli*. These mutants, long referred to as *chl* mutants (chlorate-resistant), are now renamed *mo* mutants, more accurately reflecting the role of the individual genes in molybdenum cofactor biosynthesis [7]. A summary of the current information on the biosynthetic pathway in *E. coli* is presented in Figure 1.

**Early steps in MPT biosynthesis**

The biosynthetic pathways of folate and riboflavin in plants and micro-organisms and of biopoterin in animals all utilize GTP as the initial precursor of the pterin ring [8,9]. In order to

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Abbreviations used: MGD, molybdopterin guanine dinucleotide; MPT, molybdopterin.
determine whether MPT synthesis in E. coli also entails the use of guanosine, \(^{14}C\)-labelled guanosine was added to growing cultures of the MPT-deficient E. coli mutant moeB, which accumulates large amounts of precursor Z, the final intermediate in MPT biosynthesis [10,11]. Precursor Z, which retains all ten carbon atoms present in MPT, was isolated from the cells and culture media after oxidation to the stable fluorescent pterin, compound Z, and analysed for the pattern of \(^{14}C\) incorporation. The results of studies on the synthesis of the MPT portion of the molybdenum cofactor in E. coli can be summarized as follows [11,12].

1. As in the pathways of folate, riboflavin and biotpterin synthesis, a guanosine derivative serves as the initial biosynthetic precursor of MPT, as demonstrated by the transfer of label from \([U-{\textsuperscript{14}}C]\)guanosine to precursor Z, the final MPT intermediate. This conversion appears to be mediated by the MoaA, MoaB and MoaC proteins.

2. Degradation of compound Z, the oxidation product of precursor Z, successively to pterin-6-carboxylate and pterin showed that both the ribose and guanine carbons are utilized in this reaction.

3. The C-8 carbon of the guanosine precursor is retained and incorporated as the first carbon of the MPT side chain (Figure 2.4). This aspect of MPT synthesis is distinct from all of the other pathways mentioned above, and indicates that MPT biosynthesis represents a novel route for pterin synthesis in E. coli.

Precursor Z is not detectable in moaA and moaC mu insertion mutants, implicating the products of those genes in the conversion of a guanosine compound into precursor Z. The likely involvement of the MoaB protein in the reaction has not been established because of the absence of a moaB\(^{-}\) mutant. The exact role of each protein is unknown. The amino acid sequence of MoaA contains Fe/S cluster motifs, and the recombinantly expressed protein has been shown to be a ferredoxin-like protein [13]. The presence of a folate-binding motif in the MoaB sequence [14] may be of significance, since the processing of the C-8 carbon of guanine may entail attachment to tetrahydrofolate.

**Mechanism of conversion of precursor Z into MPT**

The observation that the addition of precursor Z to crude extracts of the moaA mutant of E. coli resulted in the formation of MPT [10] led to the isolation and characterization of MPT synthase, a protein composed of two different subunits of 16 kDa (L subunit) and 8.5 kDa (S subunit), both
of which are required for the activity [15,16]. From the N-terminal amino acid sequences of the two subunits of MPT synthase, it was determined that the L and S subunits are the products of the \textit{moaE} and \textit{moaD} genes respectively [14,16].

As shown in Figure 2(B), studies with purified precursor Z and MPT synthase have shown that MPT is generated when the purified components are incubated together with no additional small molecules present [17]. The conversion of precursor Z into MPT was monitored by HPLC following the disappearance of precursor Z, and the presence of MPT in the final reaction mixture was demonstrated by conversion to the form A, form B and camMPT derivatives. The newly formed MPT was shown to remain bound to the synthase even after gel filtration.

Whereas the accumulation of precursor Z in \textit{E. coli} \textit{moeB} cells indicated the absence of active MPT synthase from the mutant, it was found that mutant cells did contain both subunits of MPT synthase, but in an inactive form [18]. In order to identify the molecular lesion in the \textit{moeB} mutant, the masses of the subunits of the active synthase from \textit{moeA} mutant and inactive synthase from the \textit{moeB} mutant were analysed by electrospray MS. The large subunits of the synthase in the two strains had identical masses; however, the small subunit isolated from the \textit{moeB} mutant had a lower molecular mass than the active enzyme by 16 Da. Since the key reaction carried out by MPT synthase is the addition of the dithiolene sulphurs to the pterin side chain, the difference in mass could be accounted for by the substitution of a sulphur for an oxygen in the active form of the synthase. A reactive transferable form of sulphur bound to the small subunit could serve as the direct sulphur source for dithiolene formation.

With MPT synthase serving as the sole source of sulphur for dithiolene formation, as studied in the purified system, the conversion of precursor Z into MPT can occur only in a stoichiometric manner. For the synthase to act catalytically, a system (MPT synthase, sulphurylase) for regeneration of the synthase-bound sulphur is required. The observation that the \textit{moeB} mutant cells contain inactive apparently desulpho MPT synthase indicated that the MoeB is in fact MPT synthase sulphurylase.

Studies on MPT synthase sulphurylase have revealed interesting features of this protein with possible mechanistic implications. The \textit{moe} locus of \textit{E. coli}, which has been cloned by Nohno et al. [19], contains two open reading frames, MoeA and MoeB. While the role of the \textit{moeA} gene product in Mo cofactor processing in \textit{E. coli} is not known, fusion genes containing segments homologous to \textit{E. coli} Mog and MoeA have been identified in \textit{Drosophila} [20], \textit{Arabidopsus thaliana} [21] and a rat synaptosomal protein termed
The relevance of gephyrin to the Mo cofactor is a moot point since the protein was isolated and characterized in an entirely unrelated contest, as a protein with high affinity for polymerized tubulin, and required for anchoring the inhibitory glycine receptor localized at central synapses [22].

The predicted amino acid sequence of MoeB, corresponding to the sulphurylase, includes a nucleotide-binding motif at the N-terminus [23]. Neutron-activation analysis and the use of specific colorimetric procedures for zinc and iron showed the presence of stoichiometric Zn in the purified protein [24]. The absorption spectrum shows a strong shoulder at 320 nm that may reflect the specific association of Zn with the protein (P. Bali and K. V. Rajagopalan, unpublished work). The 320 nm absorption band is abolished in 6 M guanidinium chloride with release of the tightly bound Zn. Release of Zn is also effected when the protein is treated with mercurials, suggesting ligation to thiol groups, probably through the Cys-Xaa-Xaa-Cys sequence that occurs twice in the MoeB sequence.

Significant sequence similarities between moeB and a number of other proteins have been identified [23]. Particularly noteworthy is that between moeB and UBAI, the gene coding for ubiquitin-activating enzyme E1. The amino acid sequences of MoeB and the yeast UBAI enzyme show 23% identity. Similar degrees of identity are noted with the activating enzymes from wheat and humans. When all three are included, the collective identity with MoeB increases to 50%, suggesting that the various UBAI genes are the products of divergence from an ancestor closely related to moeB. ATP-dependent protein-linked thioester formation is an essential aspect of the ubiquitin-dependent protein-degradation system [25] as outlined in Figure 3. The process is initiated by the attachment of the carboxylate of the C-terminal glycine of ubiquitin to a cysteine of the activating enzyme E1, forming a thioester. Ubiquitin, a highly conserved protein of 76 amino acids, bears little similarity to any of the known mo gene sequences, except that its C-terminal Gly-Gly sequence is the same as that of the MoaD subunit of MPT synthase. This similarity has led to the proposal that the active sulphur in the synthase is a thiocarboxylate at the C-terminus of the S subunit [23]. It could be postulated that a thioester is initially formed between the glycine on MPT synthase and a cysteine sulphur of the sulphurylase. Significantly, Cys-142 of the sulphurylase is conserved in all of the homologous proteins. Generation of the active thiocarboxylate of the synthase would be accomplished in a reaction involving the bound Zn and a low-molecular-mass sulphur source. This pathway of sulphur-transfer reactions may occur in other systems as well, suggested by the similarity of MoeB sequence to those of the ThiF protein in the E. coli thiamin-biosynthetic pathway [26] and the HesA protein in the nif gene region of Anabaena [27]. The ThiG1 and HesB sequences in the latter pathways respectively contain C-terminal Gly-Gly, and would be likely targets for thiocarboxylate generation.

**Dinucleotide formation**

The identification MGD in the molybdenum cofactor of Rhodobacter sphaeroides DMSO reductase [3], and subsequently in the nitrate reductase and formate dehydrogenases of E. coli [4], showed that there is yet another step in the pathway of molybdenum cofactor biosynthesis in prokaryotes. Studies on the mob mutant of E. coli have shown that the gene product of the mob locus is essential for the addition of the GMP
moiety to form MGD [28]. Wild-type cells were shown to contain both MPT and MGD, whereas mob cells contained elevated levels of MPT but no detectable MGD.

**Uptake and processing of molybdate**

The mod locus of *E. coli* encodes proteins that constitute the transport system for molybdate [29]. Thus the pleiotropic phenotype of mod mutants is abolished by increasing the molybdate content of the growth medium to 0.1–1 mM [30]. In contrast, the phenotype of the mog mutant is only partially reversed even in the presence of 10 mM molybdate [31]. A role in the attachment of Mo to the dithiolene of MPT has been proposed for the Mog protein [31]. The amino acid sequence of Mog shows remarkable similarity to that of the MoaB protein [141].

**Conclusion**

Searches of protein sequence databases have shown that Mo cofactor biosynthetic pathways similar to that in *E. coli* are present in diverse other organisms including Archaea, fungi, yeast (*Saccharomyces cerevisiae* is an exception) and *Drosophila*. Human MPT-synthetic genes are yet to be identified, but studies on human cofactor deficiency have shown that precursor Z lies in the MPT-biosynthetic pathway in humans as well [32]. The knowledge accrued from studies on Mo cofactor biosynthesis in *E. coli* should facilitate the unravelling of the cofactor-biosynthetic pathways in other systems.

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