The final stages of bacterial molybdenum cofactor (Moco) biosynthesis correspond to molybdenum chelation and nucleotide attachment onto an unique and ubiquitous structure, the molybdopterin. Using a bacterial two-hybrid approach, here we report on the in vivo interactions between MogA, MoeA, MobA, and MobB implicated in several distinct although linked steps in Escherichia coli. Numerous interactions among these proteins have been identified. Somewhat surprisingly, MobB, a GTPase with a yet unclear function, interacts with MogA, MoeA, and MobA. Probing the effects of various mo. mutations on the interaction map allowed us (i) to distinguish Moco-sensitive interactants from insensitive ones involving MobB and (ii) to demonstrate that molybdopterin is a key molecule triggering or facilitating MogA-MoeA and MoeA-MobA interactions. The results suggest that, in vivo, molybdopterin cofactor biosynthesis occurs on protein complexes rather than by the separate action of molybdopterin cofactor biosynthetic proteins.

Molybdenum is an essential trace element for most living systems including microorganisms, plants, and animals. Molybdenum is found associated with a diverse range of redox active enzymes that catalyze basic reactions in the metabolism of nitrogen, carbon, and sulfur (1). With the exception of nitrogenase, molybdenum is incorporated into proteins as the molybdopterin cofactor (Moco), an ubiquitous basic structure which contains a mononuclear Mo atom coordinated to an organic cofactor named molybdopterin (MPT) (2). The biosynthesis of Moco is an evolutionary conserved pathway that has been extensively studied in *Escherichia coli* and several genetic loci grouped together under the mo. designation (moa, moe, mob, moe, and mog) have been implicated in the pleiotropy of molybdoenzymes (3). Genes encoding highly homologous proteins implicated in Moco biosynthesis have been identified in bacteria, archaea, higher plants, and higher animals including humans. Molybdopterin cofactor biosynthesis can be divided into three stages: (i) conversion of a guanine nucleotide into the meta-stable precursor Z, (ii) conversion of precursor Z into MPT, and (iii) chelation of molybdenum by MPT, thus forming Moco. Although this compound constitutes the active form of the cofactor present in all eukaryotic and some prokaryotic molybdoenzymes, most bacterial enzymes require a modification of this basic structure to be functionally active. This modification involves attachment of a nucleotide moiety, GMP, AMP, IMP, or CMP, onto the terminal phosphate group of the MPT side chain (reviewed in Rajagopalan and Johnson (2)).

Due to its intrinsic instability, the molybdopterin cofactor has to remain bound to proteins during the whole biosynthetic process until its final delivery to apomolybdoenzymes. The crystal structure of most of the proteins involved in the biosynthetic pathway of Moco have been determined recently (4–12). Biochemical studies have indicated that newly formed MPT remains tightly bound to the MoaD-MoaE complex (MPT synthase) until its transfer to proteins able to bind it with higher affinity (13). MogA and MoeA proteins constitute such candidates and have been shown to bind MPT with distinct affinities (6, 11, 14). MPT might then be transferred from MPT synthase to MogA by direct protein interaction, and an activated molybdenum species can be subsequently inserted into the MogA-bound MPT by the aid of MoeA. The MPT-Mo cofactor can either be inserted into MPT-free apoenzymes or undergo subsequent addition of a nucleotide, for example GMP in *E. coli* by the MobA protein. However, in vitro reconstitution studies of MPT-enzymes such as sulfite oxidase have indicated that conversion of MPT to active Moco by molybdate chelation and its subsequent incorporation can be performed in the absence of MogA and MoeA (13). Similar studies performed on MGD-enzymes such as Me2SO reductase from *Rhodobacter sphaeroides* have shown that both MobB and a chaperone protein are not absolutely required for MGD insertion (15). Such observations have led us to consider alternative approaches to decipher the functions played by the mo. gene products involved in the Mo incorporation and nucleotide attachment steps.

This communication reports on the interactions existing in vivo between individual pairs of mo. gene products involved in the final stages of molybdopterin cofactor biosynthesis in *E. coli*. Effects of various mo. mutations on these interactions have been assessed and allowed the identification of protein complexes for which formation is dependent upon binding of a Moco intermediate. A comprehensive model is presented for the protein interaction network existing during the course of Moco biosynthesis in *E. coli*.

**EXPERIMENTAL PROCEDURES**

*Bacterial Strains, Plasmids, and Growth Media—*The bacterial strains and plasmids used in this work are described in Table I. *E. coli* DH5α was used as a host for plasmid constructions and maintenance. The other strains employed in this study are derivatives of *E. coli* BTH101 (16). Bacterial cultures were grown in L-broth medium under aerobic conditions. As needed, sodium molybdate or sodium tungstate was added at 1 mM final concentration. When required, 100 µg ml⁻¹ of ampicillin, 50 µg ml⁻¹ of chloramphenicol and 25 µg ml⁻¹ of tetracycline are employed.
In Vivo Interactions among mo. Gene Products

Table I

| Strain or plasmid | Description | Reference or source |
|-------------------|-------------|---------------------|
| DH5a              | supE44, ΔlacU169 (Δ88 lacZΔM15), hsdR17, recA, endA1, gyrA96, thi-1, relA1 | Lab. collection |
| BTH101            | F-, cya-99, araD139, galE15, galK16, rpsL1(Strr), hsdR2, mcrA1, mcrB1 | (16) |
| VJS720            | chd247::Tn10 (mobB247) | K.T. Shanmugan |
| VJS1780           | RK4353 mobB252::Tn10 | K.T. Shanmugan |
| VJS1778           | RK4353 mobB254::Tn10 | K.T. Shanmugan |
| BTH101moa         | BTH101 mob254::Tn10 (P1 transduction VJS1778 × BTH101) | This work |
| BTH101mod         | BTH101 mobB247·Tn10 (P1 transduction VJS720 × BTH101) | This work |
| BTH101mob         | BTH101 mobB252·Tn10 (P1 transduction VJS1780 × BTH101) | This work |

Plasmid Constructions for Two-hybrid Assays—For the generation of pT18- and pT25-derived plasmids, mogA, moa, mobA, and mobB genes were individually amplified by PCR using chromosomal DNA of strain MC4100 as template. mobA and mogA genes were amplified, KpnI-restricted, and ligated into the KpnI site of either the pT18 or pT25 plasmids. For subcloning into the pT18 vector, mogA was amplified to generate a PCR product flanked by SalI and HindIII sites. This fragment was digested and subcloned into the polylinker of the pT18 vector. For subcloning into the pT25 vector, mogA was amplified, BamHI-, SacII-restricted, and subcloned into the polylinker of the pT25 vector. Similarly, for subcloning into the pT18 vector, mobB was amplified and XhoI-restricted, whereas subcloning into the pT25 vector was performed using the Psil-Smal sites. Clones were sequenced to check for any mutation that might have been misincorporated during the amplification, and when necessary, the correct orientation of the insert was confirmed by PCR.

Two-hybrid Assays—Subcloning of PCR fragments into the pT18 vector lead to the formation of chimeric proteins with an 18-kDa carboxy-terminal fragment (225–399 amino acids) issued from the adenylate cyclase. Conversely, subcloning of PCR fragments into the pT25 vector lead to the formation of chimeric products with a 25-kDa amino-terminal fragment (1–224 amino acids) issued from the adenylate cyclase. In Indo/ ΔsacM15 mutants (BTH101) transformed with pT18 and pT25-derived plasmids. Maltose metabolism has been described (19). β-Galactosidase activity in whole cells was determined at mid-log phase with chloroform-sodium dodecyl sulfate-permeabilized cells as described by Miller (17). The β-galactosidase values presented are the average of at least three independent experiments.

RESULTS

Protein-protein interactions play a major role in most of biological processes. It is becoming evident that many proteins act in large modular structures, also referred to as protein machines or molecular networks, that are involved in specialized processes like signal transduction, metabolic cascade, and biosynthetic pathways. Taking into consideration that proteins act as modular complexes, one possible approach to uncover their respective function is to identify their potential protein partner(s). The notion behind this approach is that identification of a known partner would provide a clue to the function of the protein within a complex process. One of the most commonly used genetic systems of today is the two-hybrid system, which was originally described more than a decade ago by Fields and Song (20). This system was shown to be a powerful method for the identification of protein-protein interaction between known protein partners as well as for the identification of new partners using a library screening approach.

Interaction between E. coli NarD and NarG Can Be Detected by a Bacterial Two-hybrid System—To characterize protein-protein interactions, a bacterial two-hybrid system developed by Karimova et al. (16) and based on functional reconstitution of adenylate cyclase activity was employed. Complex formation of proteins of interest was readily ascertained by red color development of bacterial colonies on MacConkey media supplemented with maltose and by an elevated β-galactosidase activity. As the two measurements gave corroborating results, only the enzyme activities are presented. A positive control, pT25-Zip/pT18-Zip, which contains the sequence for a 35-amino acid leucine zipper motif of GCN4 fused to both pT25 and pT18, was tested for interaction (16). Under our assay conditions, pT25-Zip/pT18-Zip exhibited a high level of adenylate cyclase complementation (intense red color on MacConkey-maltose indicator plates in 48 h; 1500 Miller units of β-galactosidase activity).

Enzyme Assays—Nitrate reductase activity was assayed by procedures already described (19). β-Galactosidase activity in whole cells was determined at mid-log phase with chloroform-sodium dodecyl sulfate-permeabilized cells as described by Miller (17). The β-galactosidase values presented are the average of at least three independent experiments.

Experimental Procedures—Transduction with P1 phage was performed as described by Miller (18). Using BTH101 as a recipient strain, BTH101mob254·Tn10, BTH101mobB247·Tn10, and BTH101mobB252·Tn10 were obtained. Mapping of the mutations was confirmed by phenotype complementation analysis.

Enzyme Assays—Nitrate reductase activity was assayed by procedures already described (19). β-Galactosidase activity in whole cells was determined at mid-log phase with chloroform-sodium dodecyl sulfate-permeabilized cells as described by Miller (17). The β-galactosidase values presented are the average of at least three independent experiments.
action between NarJ and NarG proteins that correspond to a specific chaperone type protein and the catalytic subunit of the membrane-bound nitrate reductase A, respectively. These proteins form a tight complex during the maturation of the E. coli nitrate reductase A (21). Dissociation of this complex only occurs when the molybdenum cofactor has been inserted into the NarG catalytic subunit. narJ was cloned into the bait plasmid pT18, and narG into the prey plasmid pT25. High levels of β-galactosidase were observed when both plasmids were present in the recipient cell, BTH101 (intense red color on MacConkey-maltose indicator plates within 48 h; 1200 Miller units of β-galactosidase activity). No activity was observed when either fusion plasmid was used separately or when used with Zip-derived plasmids (cells remain white on MacConkey-maltose plates after several days; 50 Miller units). When NarH corresponding to the electron transfer subunit was used with the NarJ fusion protein, no activity was detected (data not shown) as shown previously by BIACORE analysis (21). Thus, this approach allows the detection of specific interaction between NarJ and NarG. Moreover, endogenous NarG issued from the recipient strain BTH101 cannot mediate interaction between NarJ and NarH fusion proteins using the two-hybrid system. These results set the stage for analyses of the interactions existing in vivo between mo. gene products described below.

**Defining the Interactions Existing in Vivo in a Wild-type Strain, BTH101**—To study the interactions existing in vivo among proteins involved in the final stages of Moco biosynthesis, the following genes, moea, mogA, mobA, and mobB were subcloned individually in both pT18 and pT25 plasmids. In an initial control, the functionality of each of the fusion proteins with MoeA, MogA, and MobA were tested by phenotype complementation analysis. Due to the lack of phenotype of mobB mutant, functionality of the corresponding fusion proteins has not been assessed. On the other hand, complete restoration of the phenotype has been systematically observed by measurement of nitrate reductase activity (> 95% activity of the wild-type strain) indicating that all of the tested plasmid constructions produce active fusion proteins despite the presence of T25 or T18 domains at the amino terminus or carboxyl terminus, respectively. Further control experiments were carried out in which each of the mo. fusion constructs were tested with either pT18-Zip or pT25-Zip. None of the transformants exhibited adenylate cyclase complementation (cells remain white on MacConkey-maltose plates after several days; 50 Miller units), demonstrating recognition specificity displayed by the Mo. fusion proteins (data not shown).

Fig. 1A displays results obtained from complementation between the Mo. chimeric proteins. To discriminate false-positive from true interactions, each of the interacting pairs have been tested in both directions and have given similar results on both indicator plates and β-galactosidase measurements. Interestingly, MoeA interacts with both MogA and MobA to result in significant complementation (Fig. 1A, lanes 1–2), whereas MogA fusion proteins failed to interact with MobA (Fig. 1A, lane 3). Somewhat surprisingly, MobB interacts with MoeA, MogA, and MobA to varying extents (Fig. 1A, lanes 4–6). As a representative control, MoeA does not interact with the Zip domain (Fig. 1A, lane 7).

One can take advantage of this two-hybrid system in verifying the multimerization ability of each of the tested Mo. proteins. Crystal structures and biochemical experiments have indicated that MoeA exists as a dimer (10, 11) and MogA as a trimer (6), whereas MobA is monomeric (4, 5). MobB exists as a dimer according to gel filtration experiments (22). Transformants with pT25-Moba/pT18-Moba exhibit a basal level of β-galactosidase activities supporting a monomeric state of MobA (Fig. 2, lane 1). In contrast, cotransformation with either pT25-MobB/pT18-MobB or pT25-MoeA/pT18-MoeA leads to a very high level of adenylate cyclase complementation (Fig. 2, lanes 2–3) supporting a dimeric state of both MobB and MoeA. Finally, no adenylate cyclase complementation was observed when using MogA chimeric proteins (Fig. 2, lane 4).

**Probing the Effect of Moco Absence on the Interactions**—Some of the visualized interactions might correspond to transient complexes occurring during Moco biosynthesis. At least, two of the tested proteins MogA and MoeA being able to bind Moco intermediates with various affinities (6, 11, 14), one can envision that Moco intermediates trigger or facilitate some protein interactions. To assess the effect of complete absence of Moco on the interactions existing among the four tested proteins, we constructed by P1 transduction a BTH101moa::Tn10 mutant affected at the first stage of Moco biosynthesis (See “Experimental Procedures”). Fig. 1B displays results obtained from adenylate cyclase complementation between the Mo. chimeric proteins in the Moco-deficient strain. Similar complementation levels were reached with MobB fusion proteins in the BTH101moa strain in comparison to the wild-type strain BTH101 (Fig. 1B, lanes 4–6). Interestingly, transformants expressing either MobA-MoeA or MogA-MoeA chimeric protein pairs exhibited greatly reduced levels of β-galactosidase activities (Fig. 1B, lanes 1–2) leading to the assumption that Moco binding onto these proteins allows them to interact in a wild-type cell. The crystal structure of the MoeA dimer has not
revealed the presence of any cofactor (11). Accordingly, absence of Moco has no effect on the complementation level observed in cotransformants expressing T25MoeA/T18-MoeA proteins (data not shown).

Probing the Effect of Molybdenum Deficiency on the Interactions — To identify the nature of Moco intermediates that trigger or facilitate protein-protein interactions between MogA-MoeA and MoeA-MobA, the effect of molybdenum deficiency has been assessed. In a mod mutant, Moco biosynthesis is arrested at an intermediate step resulting in the sole presence of MPT in the absence of active Moco for both MPT- and MGD-containing enzymes. The introduction of high levels of molybdate into the growth medium, however, restores molybdenum activities to mod strains. The interactions have thus been evaluated in the strain BTH101modB::Tn10 constructed by P1 transduction. No significant differences were obtained in comparison to the wild-type BTH101 strain (Fig. 3) indicating that the sole presence of MPT is sufficient to allow MogA-MoeA and MoeA-MobA interactions.

Tungstate is a close analog of molybdate and has been shown to be incorporated via an unknown mechanism into the Moco biosynthetic pathway. Growth in the presence of tungstate gives rise to a biologically inactive tungsten form of the Moco that leads to the formation of inactive forms of most molybdoenzymes (24–26). However, such a cofactor can be incorporated into E. coli trimethylamine-N-oxide reductase (27) and most probably into periplasmic nitrate reductase (28). The addition of tungstate to the growth medium for strain BTH101mod gave rise to some specific differences in comparison to the wild-type strain (Fig. 3). Among the tested interactions, only MoeA-MogA and MoeA-MobA were significantly enhanced under these conditions (Fig. 3, lanes 1–2). The most likely explanation is that tungstate leads in such strain to the formation of a tungsten form of cofactor that is unable to be incorporated efficiently to mostly resident apomolybdoenzymes such as membrane-bound nitrate reductases (24). Consequently, one could envision accumulation of Moco intermediates (MPT and MPT-Mo) for MPT-containing enzymes but defective in the GMP attachment step (29, 30). In such a strain, presence of either T18-MobA or T25-MobA fusion proteins leads to a full restoration of the phenotype revealed by nitrate reductase activity measurement (data not shown). Hence, interactions involving MobA have not been tested. No significant differences in the interaction map were obtained in comparison to the wild-type BTH101 strain (data not shown) indicating that the metal has no effect on the interactions as the sole presence of either MPT in a mod strain or MPT-Mo in the mob strain is sufficient to allow the tested interactions.

**DISCUSSION**

Specific protein-protein interactions are central to most biological processes and probably in molybdenum cofactor biosynthesis as well. A bacterial two-hybrid approach (16) was chosen here, rather than the yeast system, as it could find numerous applications for in vivo analysis of newly identified bacterial protein interactions and become an essential complementary tool to biochemical studies currently performed on Moco biosynthetic proteins.

The multimerization of MogA, MoeA, MobA, and MobB proteins has been assayed. Complete agreement has been observed between our results and published data for dimeric proteins (MoeA and MobB) and for a monomeric protein (MobA). In contrast, an apparent discrepancy has been observed for MogA, reported to be a trimer (6), and for which no complementation was obtained by the two-hybrid assay. The most likely explanation is a steric hindrance within the T18-MogA/T25-MogA heterodimer in placing in close proximity the Cya domains. Indeed, the carboxyl terminus of each subunit within the MogA trimer are located on the same side and at the opposite of the amino-terminal ends avoiding close contact between the Cya domains. Consequently, the interaction between the MogA chimeric proteins will not lead necessarily to adenylate cyclase complementation. Importantly, one has to mention that high complementation levels reached in the case of MobB or MoeA dimers suggest the formation of extremely stable complexes. Conversely, lower complementation levels obtained with other interacting pairs most likely result from transient complexes that might require additional factors to be stabilized.

Multiple pairwise interactions have been identified including some unexpected ones such as those involving MobB as summarized in Fig. 4. Indeed, MobB, a GTPase protein with a yet unclear function (22), interacts with each of the tested Mo. proteins. Although no role was observed for MobB in the in vitro activation of Me₂SO reductase (15), it enhanced the activation of nitrate reductase, another MGD-containing enzyme (31). Such observations stimulated our interest in the role played by MobB and led us to reconsider its possible role in Moco biosynthesis. Examination of the mechanisms of metalocenter assembly has revealed the strictly conserved involvement of a protein with a nucleotide-binding motif (UreG, HypB, CooC) (32–34). Interestingly, UreG has been shown to interact with several proteins required for the maturation of urease, a nickel enzyme (32, 35, 36). By analogy, MobB would interact with several Mo. proteins to facilitate Moco insertion. Experiments are in progress to see whether the loss of GTPase activity of MobB would affect its ability to interact.

MogA and MoeA form a tight complex in vivo. It has been shown that prokaryotic moeA and mogA gene sequences are systematically fused into a single open reading frame in eu-karya-like plants, fungi, *Drosophila*, or higher eu-karya-like humans (37–40). It is therefore highly probable that these two bacterial proteins, which are involved in a common biosynthetic step of the Moco, interact with each other. However, in vitro mixing of purified MoeA and MogA proteins under differ-
ent conditions did not form detectable complexes, leading to the assumption that either these proteins do not interact or that a factor was missing under these conditions (10). Hence, the effect of mo. mutations interrupting the Moco biosynthetic pathway at different levels has been probed on the interaction map. Such experiments performed in the BTH101 moa::Tn10 strain allowed to identify and distinguish, for the first time, two classes of interactants, i.e. those that require the presence of Moco from those that do not. Whereas interactions involving MobB are not sensitive to the absence of Moco, MogA-MoeA and MoeA-MobA interactions are strongly affected supporting the view that Moco intermediates facilitate complex formation and/or stabilization. Molybdenum deficiency obtained in a mod mutant as well as a mob mutation impairing MGD synthesis has no effect indicating that MPT is a key molecule triggering or facilitating some specific protein-protein interactions. Interestingly, tungstate addition to the growth medium of a mod strain brings about an activation of complementation for both MogA-MoeA and MoeA-MobA interacting pairs of some 2-fold over that found in its absence. Accumulation of these Moco-dependent protein complexes is most certainly a consequence of the accumulation of Moco intermediates (MPT and MPT-W) under these conditions (41).

Overall, such observations are in full agreement with current knowledge on the function of MogA, MoeA, and MobA. Chelation of molybdenum by MPT requires the concerted action of MogA and MoeA (42, 43). Our data provide the evidence that a MogA-MoeA complex can only occur in presence of MPT. In eukarya, such a complex is naturally occurring via the existence of a MogA-MoeA fusion protein (37, 39, 40). The resulting MPT-Mo-loaded MogA-MoeA complex might constitute the Moco donor to MPT-free apoenzymes in prokaryotes. Finally, in bacteria a nucleotide has to be attached to MPT with the help of MobA resulting in the MGD cofactor, the active cofactor for most prokaryotic molybdoenzymes. One can make the assumption that the preceding MogA-MoeA complex delivers MPT-Mo to MobA via the formation of a transient complex composed at least of MoeA and MobA. Here again, according to our results such a complex would exist only in presence of Moco. Although the final steps of bacterial Moco biosynthesis have not been clearly defined, the processes of Mo insertion and of dinucleotide attachment seem to be strongly linked and our data provide a further argument to this assumption. Finally, a minimal complex composed of MoeA and MobA might be involved in MGD transfer to the apoenzymes. Such a complex delineation is reminiscent of the Moco carrier linking Moco biosynthesis to its subsequent incorporation into various apomolybdoenzymes. In E. coli, Moco has been shown to be protected by tight binding to a 40-kDa Moco carrier protein (41). A similar situation has been encountered in Chlamydomonas reinhardtii with a 50-kDa carrier protein (44, 45). However, little is known concerning the identity of this carrier molecule.

FIG. 3. Effect of mod mutation and tungstate addition on the interaction map. Black bars represent values obtained from the wild-type strain, BTH101. Light gray bars represent values obtained from the mod mutant, BTH101modB::Tn10. Dark gray bars represent values obtained after tungstate addition (1 mM) to the growth medium of the mod mutant. β-Galactosidase activity is expressed in Miller units.

FIG. 4. Schematic representation of the interaction network between MogA, MoeA, MobA, and MobB. A, in BTH101 strain able to synthesize Moco, B, in BTH101moa::Tn10 strain impaired in Moco synthesis. The arrow indicates the interaction. The asterisk indicates proteins able to dimerize.
In Vivo Interactions among mo. Gene Products

than by the separate action of different Mo. proteins. A complex of Moco biosynthetic proteins also functioning as the Moco carrier could thus ensure Moco protection. To validate this model, a number of questions have to be answered. The most relevant one is verifying whether one or several mo. gene products involved in the final stages of Moco biosynthesis such as MoaA and MoB can readily interact with apomolybdoenzymes. The bacterial two-hybrid approach described here constitutes an essential tool to address such a fundamental question.

Based upon the conserved fusion event occurring between eukaryotic MogA and MoaE and on the observed interaction between E. coli counterparts in the presence of Moco, one can conclude that it becomes important to facilitate substrate-product flow by the existence of a Moco-biosynthetic multienzyme complex. Formation of such complexes would ensure both the fast and protected transfer of oxygen-sensitive intermediates within the reaction sequence from MPT to active Moco and its subsequent delivery to resident apomolybdoenzymes. The channeling of substrates is a well known mechanistic process for the direct delivery of a reaction intermediate from the active site of one enzyme to the active site of a second enzyme without prior dissociation into the bulk solvent (see for review Refs. 46, 47). There are continuing reports on the existence of complexes of sequential metabolic enzymes (48–51). These systems share common features such as the existence of multiple and separate active sites connected or not via a molecular tunnel and coordination of the individual reactions through allosteric coupling of one active site with another. Interestingly, whereas MPT binding studies on the MogA-like G domain of Cnx1 revealed single independent high affinity binding sites on each monomer in the trimer, MoaE-like D domain binds MPT with a lower affinity and in a cooperative manner (14, 40). Solving the three-dimensional structure of eukaryotic MogA-MoaE fusion protein would most certainly provide information concerning the occurrence or not of a substrate-channeling mechanism by locating each of the active sites.

In summary, this report describes the first evidence for in vivo protein-protein interactions among the mo. gene products involved in the final stages of Moco biosynthesis in E. coli. In addition, the present work extends previous knowledge on the function of MogA, MoaE, MoaB, and MscB by providing evidence that they display specific pairwise interactions and that some of these interactions require the binding of Moco intermediates to these proteins. Each interacting pair reflects a potential mechanism by which Moco intermediates interact with various proteins in the actual cellular environment, and as such, must be studied to achieve an unified mechanism that explains the numerous interactions existing between these mo. gene products. The results of the present study suggest that an additional level of complexity may be found in the mechanism of Moco biosynthesis and incorporation into the apoenzymes.

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REFERENCES

1. Hille, R. (1996) Chem. Rev. 96, 2757–2816.
2. Rajagopalan, K. V., and Johnson, J. L. (1992) J. Biol. Chem. 267, 10199–10202.
3. Rajagopalan, K. V. (1996) in Escherichia coli and Salmonella typhimurium (Neidhardt, F. C., ed), pp. 674–679, ASM Press, Washington DC.