Molybdenum Enzymes and Molybdenum Cofactor in Mycobacteria

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ABSTRACT

When intracelluar pathogens enter the host macrophages where in addition to oxidative and antibiotic mechanisms of antimicrobial activity, nutrients are deprived. Human pathogen *Mycobacterium tuberculosis* is one of macrophage parasitisms, which can replicate and persist for decades in dormancy state in virulent environments. It is very successful in escaping the killing mechanisms of macrophage. Molybdenum (Mo) enzymes involve in the global carbon, sulfur, and nitrogen cycles by catalyzing important redox reactions. There are several Mo enzymes in mycobacteria and they exert several important physiological functions, such as dormancy regulation, the metabolism of energy sources, and nitrogen source. Pterin-based Mo cofactor (Moco) is the common cofactor of the Mo enzymes in mycobacteria but the cofactor biosynthesis is nearly an untapped area. The present article discusses the physiological function of Mo enzymes and the structural feature of the genes coding for Moco biosynthesis enzymes in mycobacteria. J. Cell. Biochem. 112: 2721–2728, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: MYCOBACTERIA; MOLYBDENUM ENZYMES; MOLYBDENUM COFACTOR

Mycobacteria are important microbes residing in wide niches, which include saprophytic soil bacteria like *Mycobacterium smegmatis* and pathogens such as *Mycobacterium leprae* and *Mycobacterium tuberculosis.* The latter is a pathogen responsible for an estimated 8 million new infections and claiming nearly 2 million lives annually [Dye et al., 2005]. It is extremely difficult to eradicate this pathogenic bacterium, because it can transmit by respiratory route, and invade macrophages where it can replicate and persist for decades in dormancy state prior to reactivation. Further more, treatment of tuberculosis is impeded by the emergence of multi- and extensively-drug resistant strains and the synergism with human immunodeficiency virus (HIV). More profound understanding of the molecular biology and physiology of mycobacteria is imperative to combat against this bug.

It is known to all that the molybdenum (Mo) is an essential microelement for nearly all organisms. Molybdenum as molybdate is bioavailable. When taken up by cells, molybdate is incorporated by complex biosynthetic process into metal cofactors [Schwarz, 2005]. There exist two disparate cofactors to handle the redox state and catalytic power of molybdenum. One type is pterin cofactor, which is pterin derivative, termed molybdopterin or metal-binding pterin (MPT), with a C6-substituted pyrano ring, a terminal phosphate and a unique dithiolate group binding Mo (Fig. 1). Iron-Mo cofactor is the other type of Mo-containing cofactor exclusively found in nitrogenase [Allen et al., 1994; Hille, 1996].

Similar to most microorganisms, *M.tuberculosis* requires Mo for essential biological function. In this review, we give a short review of pterin cofactor-contained Mo enzymes and cofactor in mycobacteria, discussing the potentially biological function of Mo enzymes.

MOLYBDENUM ENZYMES IN MYCOBACTERIA

In nature, there are more than 40 bacterial Mo enzymes [Hille, 1996; Kisker et al., 1997; Rothery et al., 2008]. However, there are a few Mo enzymes studied in mycobacteria. The physiologic function of Mo enzymes in mycobacteria will be discussed later.

NITRATE REDUCTASE

When *M. tuberculosis* encounters macrophages, phagocytosis of the tubercle bacilli appears. The tubercle bacilli phagocytized will face extremely dangerous environments, because in addition to oxidative and antibiotic mechanisms, macrophages are able to
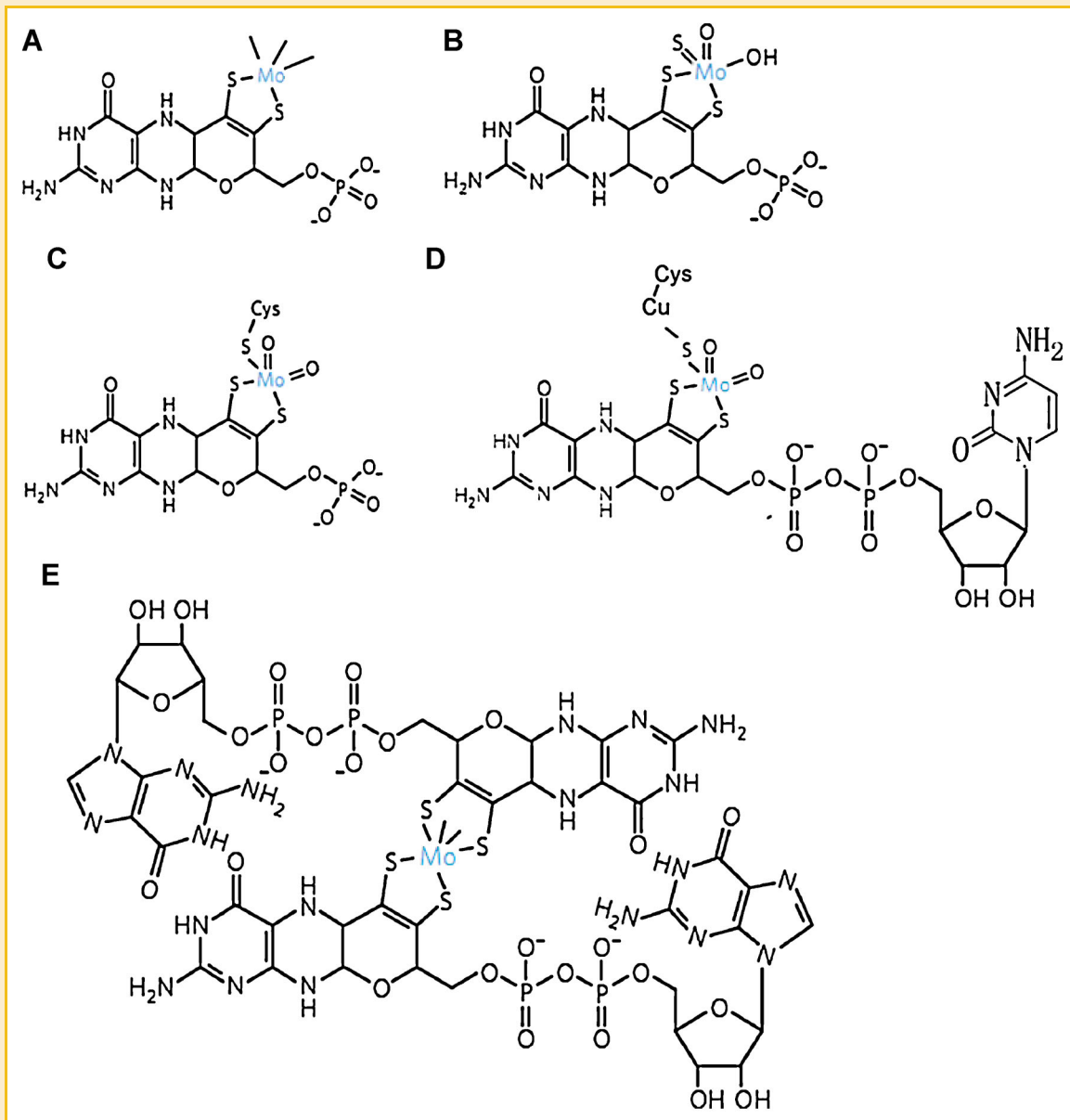
Fig. 1. The structures of molybdenum cofactors. Structure of molybdenum cofactor (A), Moco from enzymes of the xanthine oxidase (B), sulfite oxidase families (C) and carbon monoxide dehydrogenase (D), and Mo–bis–MGD (E). Figures are drawn based on Schwarz [2005] and Schwarz et al. [2009].

deprive intracellular pathogens of required nutrients [Appelberg, 2006]. Then, Granulomas formed and resulting lesions are usually able to control replication and limit the access of nutrients such as oxygen. Thus *M. tuberculosis* has only limited access to nutrients in infected tissue [Munoz-Elias and McKinney, 2006]. Fortunately, *M. tuberculosis* can use nitrate as nitrogen and energy source. Because nitrate is available in infected tissue, it is generated spontaneously from nitric oxide, which is the product of nitric oxide synthase [Bogdan, 2001]. Studies have found that *M. tuberculosis* has an *narGHJI* locus able to produce nitrate reductase activity [Weber et al., 2000], which mediates the respiratory [Sohaskey and Wayne, 2003] and assimilatory [Malm et al., 2009] reduction of nitrate.

Profuse evidences suggest that the nitrate reductase system is an important virulence factor for *M. tuberculosis* and *M. bovis*. Nitrate enhances the survival of *M. tuberculosis* during inhibition of respiration [Sohaskey, 2008] and elevated levels of nitrate reductase activity increase the virulence [Goh et al., 2005]. A *narG* knockout mutant of BCG showed depressed virulence and reduced lung damage in severe combined immunodeficiency mice [Weber et al., 2000; Fritz et al., 2002]. Additionally, transcripts of *narG* and the *narK2X* operon were detected in granulomas from the lungs of tuberculosis patients [Fenhalls et al., 2002; Rachman et al., 2006]. These results suggest that nitrate reductase plays a significant role in survival and virulence of *M. tuberculosis* and *M. bovis* in host cell.

Interestingly, *M. tuberculosis* expresses constitutively *narGHJI*. The process of nitrate reduction, however, is regulated via NarK2 and NitBD. Under reducing conditions, the transporter NarK2 elevates the concentration of intracellular nitrate by transporting it into cell [Sohaskey and Wayne, 2003; Sohaskey, 2005]. Under nitrogen-limiting conditions, the GlnR, a product of response regulator gene *glnR*, acts as a transcriptional activator of *nitBD*, and regulates *nirBD* expression, which encodes nitrite reductase mediating the assimilatory reduction of nitrite [Malm et al., 2009]. So, mycobacteria probably regulate the nitrate reduction by increasing the concentration of nitrate and removing the reductive product of nitrate, not by controlling the expression of nitrate reductase.

CARBON MONOXIDE DEHYDROGENASE

Carbon monoxide dehydrogenase (CO-DH) catalyzes the oxidation of CO to CO_2 in carboxydobacteria, which grow aerobically with CO as the sole source of carbon and energy. Recently, Park et al. [2007] have demonstrated that the CO-DHs from several mycobacteria possess nitric oxide dehydrogenase (NO-DH) activity and may exert function in the protection of mycobacterial pathogens from nitrosative stress during infection. Cloning and sequencing of the genes of *Mycobacterium* sp. strain JC1 DSM3803 revealed the presence of duplicated sets of genes for three subunits of the enzyme, *cut B1C1A1* and *cutB2C2A2*, in operons and a cluster of genes encoding protein involved in CO metabolism and a possible transcriptional regulator. Primer extension analysis indicated that there are two promoters one induced in the presence of CO and the other expressed in the absence of CO [Song et al., 2010].

It is known to all that *M. tuberculosis* infection of macrophage cells significantly increases the expression degree, protein levels, and enzymatic activity of heme oxygenase-1, which can produce CO [Kumar et al., 2008]. Present papers reported that CO is a *M. tuberculosis* signal capable of inducing the dormancy (Dos) regulon [Kumar et al., 2008] and may represent a general cue used by pathogens to sense and adapt to the host environment [Shiloh et al., 2008]. These findings suggest that CO produced by host can activate the dormancy regulon during infection. Interestingly, due to the characteristic of the promoters of CO-DH, we can suppose mycobacteria have the capability to express the CO-DH during infection, and resulting CO-DH will catalyze the oxidation of CO to CO_2. So the concentration of CO will depress and the one of CO_2 will be elevated. Elevated concentration of CO_2 is essential for the growth of tubercle bacilli especially under acerbic environment [Schaefer et al., 1955], which can diminish the dependence on biotin for the assimilation of CO_2. All these results will induce tubercle bacilli reactivation and not dormancy. Probably, there are some mechanisms to maintain the balance between dormancy and reactivation. It is a potential path to explore the mechanisms that control the physiological state of mycobacteria in infected tissue.

BIOTIN SULFOXIDE REDUCTASE

Biotin plays a significant role in the citric acid cycle, cell signaling, epigenetic regulation of genes, and chromatin structure [Zempleni, 2005]. It not only assists in various metabolic reactions but also helps to transfer carbon dioxide. The synthesis of notorious long-chain mycolic acids, which are a major component of mycobacterial cell envelope of characteristic lipids is originated by the carboxylation of acetyl coenzyme A to yield malonyl-CoA. This reaction is carried out by the biotin-dependent enzyme acetyl-CoA carboxylase, which is a class of enzymes, which in addition to fatty-acid biosynthesis, are important for gluconeogenesis as well as propionate catabolism [Knowles, 1989]. These facts suggest that biotin is very important to *M. tuberculosis*. Research has discovered the complete biotin biosynthesis pathways in many different microbes, but the study on the biotin biosynthesis in *M. tuberculosis* is exiguous. Although the biotin synthesis genes in *M. tuberculosis* were deciphered [Cole et al., 1998; Camus et al., 2002] and some products of these genes were investigated [Bhor et al., 2006; Mann and Ploux, 2006], the process of biotin biosynthesis remains an unknown quantity.

Biotin is unstable and spontaneously oxidizes to biotin sulfoxide. The *Escherichia coli* biotin *d*-sulfoxide reductase reduces biotin sulfoxide to biotin [del Campillo-Campbell and Campbell, 1982] and has essential role in assimilation of free oxidized methionines in *E. coli* [Ezraty et al., 2005]. The fact that mutants unable to synthesize biotin can use biotin *d*-sulfoxide as their sole source of biotin [Melville, 1954] suggests that reduction of biotin *d*-sulfoxide is one way of regeneration of biotin. Probably, this way can save energy sources and resources in extreme environment. Additionally, due to biotin sulfoxide, reductase catalyzes the reduction of biotin *d*-sulfoxide to *d*-biotin, it has been postulated that it is a potential protector of the cell from oxidative damage similar to the proposed roles of methionine sulfoxide reductase [Ejiri et al., 1980; Brot et al., 1981; Rahman et al., 1992; Ezraty et al., 2005] and superoxide dismutase [Fridovich, 1989]. Oxidative mechanism is one of antimicrobial activity of macrophage, so escaping from oxidative damage is tubercle bacilli's special skill. When facing oxidative environment, the tubercle bacilli must try its best to adapt to it. With respect to the genes, the homologous genes *bisC,* which encodes biotin *d*-sulfoxide reductase, was found in *M. smegmatis*, *M. tuberculosis*, and *M. bovis*. However, presently there is no report on whether the homolgous gene *bisC* has the capability to encode active protein, the function of the gene *bisC* need to be investigated in the future.

THE OTHER MOLYBDENUM ENZYMES

The initial step of degradation of many pyridine derivatives is a nucleophilic attack in *ortho*-position to the heteroatom, which is catalyzed by molybdenum enzymes. The degradation of isonico-tinate (pyridine-4-carboxylate) in mycobacteria proceeds via the formation of 2-hydroxyisonicotinate and 2,6-dihydroxypyridine-4-carboxylate[Kretzer et al., 1993]. Isonicotinate dehydrogenase catalyzes the formation of 2-hydroxyisonicotinate from isonicoti-nate, and then the 2-hydroxyisonicotinate is oxidized to 2,6-dihydroxypyridine-4-carboxylate by 2-hydroxyisonicotinate dehydrogenase. The two dehydrogenases were purified to apparent

homogeneity and were characterized as members of the molybdenum-containing dehydrogenases of the xanthine oxidase family [Kretzer et al., 1993; Schrader et al., 1998].

Additionally, in the facultative methylotrophic bacterium *Mycobacterium uaccae* 10, there are two inducible alternatively NAD^+-dependent formate dehydrogenases: FDH I and FDH II. In the presence of molybdenum, FDH I is induced; under conditions of molybdenum deficiency (tungsten in the medium), FDH II is activated [Karzanov et al., 1991].

MOLYBDENUM COFACTOR

The Mo cofactor forms active site of all Mo enzymes. Mo cofactor consists of FeMo-co and pterin-based cofactor. In this review, we will only discuss the pterin-based cofactor and the term Moco will represent the pterin-based cofactor. Moco is synthesized by a conserved biosynthetic pathway divided into four steps on the basis of the biosynthetic intermediates: cyclic pyranopterin monophosphate (cPMP), MPT, and MPT-AMP (Fig. 2). The
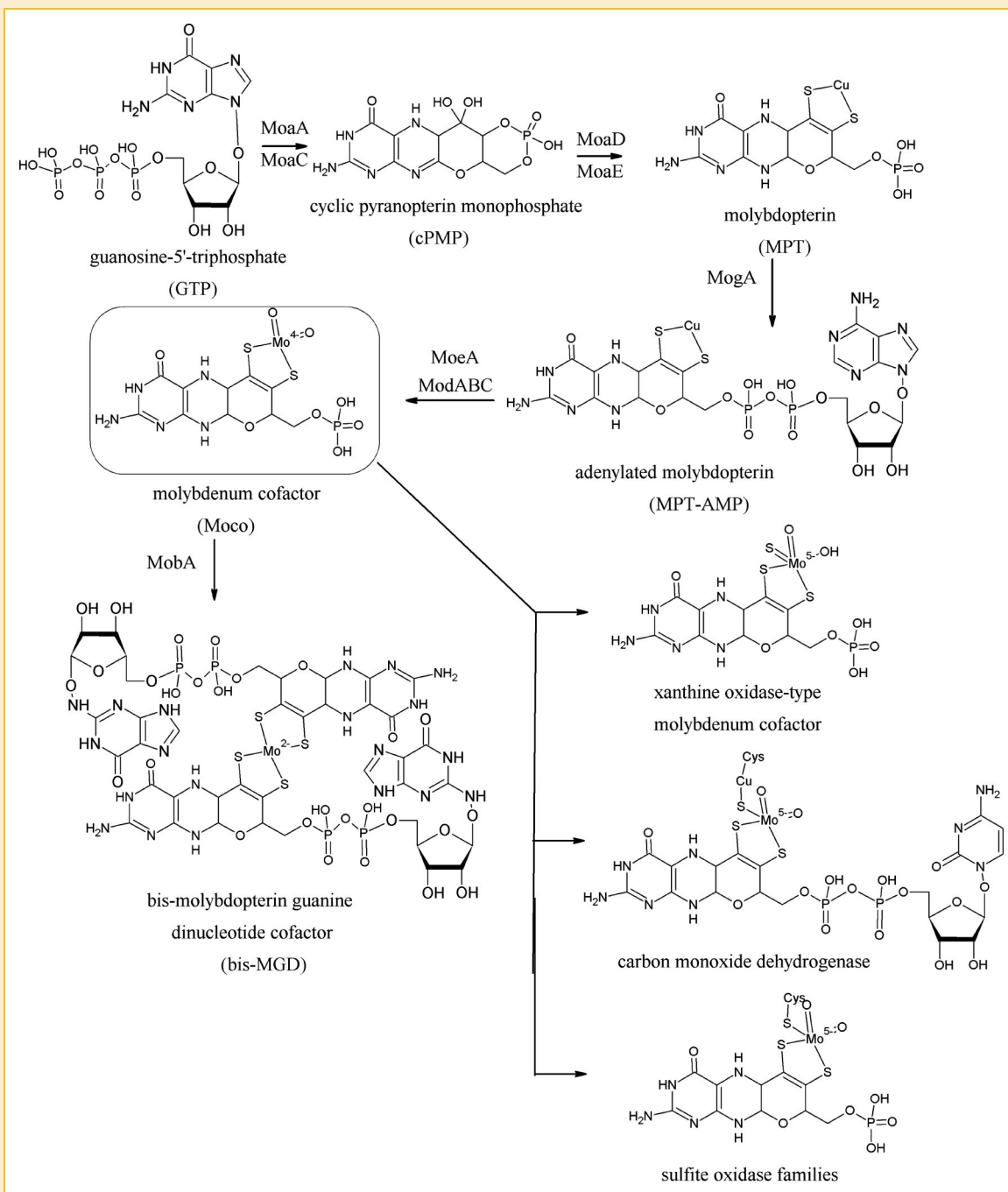

Fig. 2. Pathway for molybdenum cofactor biosynthesis in bacteria [Schwarz, 2005]. The abbreviated designations close to the arrow are proteins involved in Moco biosynthesis.

biosynthetic pathway has been summarized in detail [Reiss and Johnson, 2003; Schwarz, 2005; Schwarz and Mendel, 2006]. In *E.coli*, there are *moa*, *mob*, *mod*, *moe*, and *mog* altogether five Moco specific operons (more than 15 genes) involved in cofactor biosynthesis [Shanmugam et al., 1992; Schwarz, 2005; Schwarz and Mendel, 2006; Schwarz et al., 2009]. The function of all of these operons in *E.coli* is well understood [Shanmugam et al., 1992; Schwarz, 2005; Schwarz and Mendel, 2006; Schwarz et al., 2009], however, mycobacterial Moco biosynthesis remains an untapped area.

Although there are homologous genes coding for Moco biosynthesis enzymes in *M. tuberculosis* [Cole et al., 1998], *M. bovis* [Garnier et al., 2003], *M. abscessus* [Ripoll et al., 2009], *M. marinum* [Stinear et al., 2008], *M. ulcerans* [Stinear et al., 2007], *M. avium subspecies paratuberculosis* [Li et al., 2005], the activity of these genes are not biochemically identified.

However, not like in *E. coli*, only *moa* and *mod* locus in mycobacteria are organized in a cluster together, for the other genes in mycobacterial genomes are dispersed. For *moa* locus, *M. tuberculosis* and *M. bovis* have a locus (Fig. 3A), which probably forms operon, because *moaABCD* genes are organized in a cluster together and the arrangement of all *moaABCD* genes is similar to the one of *E. coli*. The fact that the Rv 3124 is a positive regulator of Moco biosynthesis [Mendoza Lopez et al., 2010] suggests that this locus is probably an operon. Interesting, the *moaE* gene of *M. tuberculosis* is separated by some other genes, which include the *moeB*; however, in genome of *M. bovis*, the *moaE* gene is lost. While the other locus (Fig. 3B) is aberrant in direction and order compared with the ones of *E. coli*. This locus is potentially not an operon in *M. tuberculosis*, *M. bovis*, and *M. avium ssp. Paratuberculosis K-10*.

In addition, there is another *moa* locus (Fig. 3C) in *M. tuberculosis* and *M. bovis*. At this locus in *M. tuberculosis*, there are only *moaC3* and a *moaD-moaE* fusion gene *moaX*. Unlike *M. tuberculosis*, *M. bovis* has *moaA3B3C3* gene and *moaX* gene, which encodes MoaD-MoaE fusion protein. These genes in *M. bovis* organize in a cluster together and probably form active operon.

For *mod* locus, there are three genes coding for Mo ABC transport system (ModABC) and a regulative gene encoding a repressor protein ModE. In all organisms studied so far, molybdate transport genes are regulated by a repressor protein ModE [Grunden and Shanmugam, 1997]. Homologs of the *modABC* were found in all mycobacterial genomes except in *M. leprae*, but the homologs of the *modE* was not found in all mycobacterial genomes (Fig. 4). In *M. smegmatis*, there is a ModE variant, Mop/Di-Mop proteins (Fig. 4) [Zhang and Gladyshev, 2008]. However, currently there is no conclusion regarding the functions of ModE variants. In addition, the phenomenon that almost half of ModABC-containing
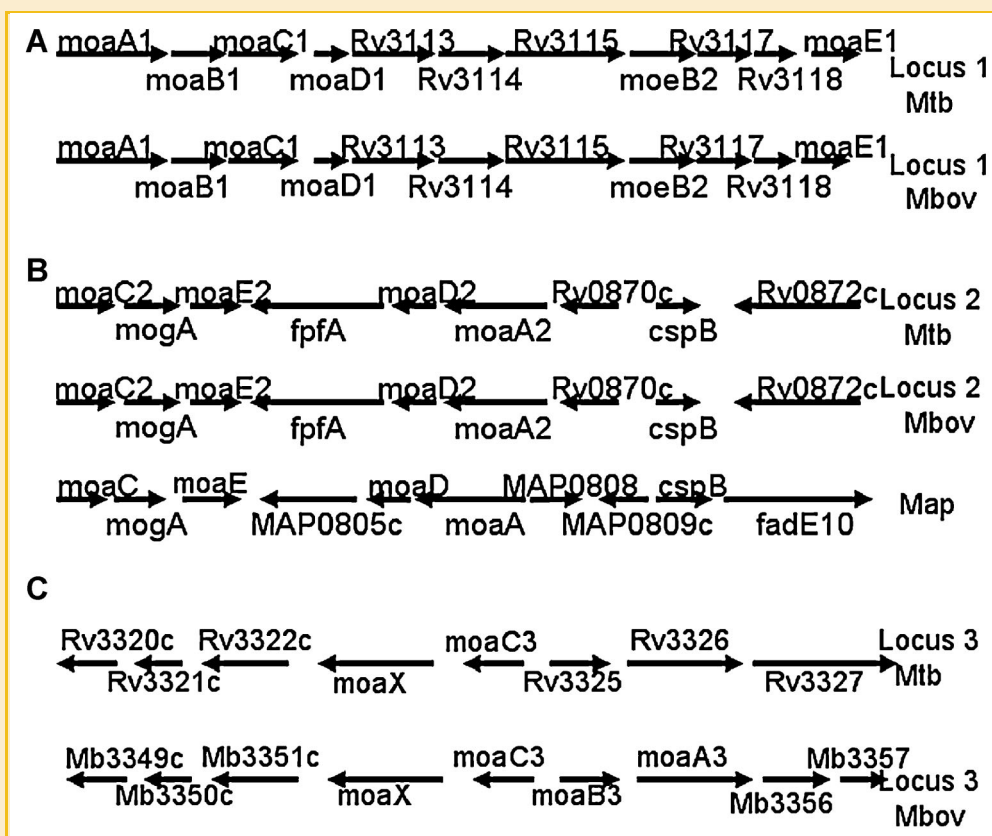

Fig. 3. Construction of *moa* locus across mycobacterial genomes. Arrows indicate the orientation of open reading frames. Original genome annotations have been listed, and the names of organisms have been abbreviated as follows: Mtb, *Mycobacterium tuberculosis*; Mbov, *Mycobacterium bovis*; Map, *Mycobacterium avium* ssp.*paratuberculosis K-10*.
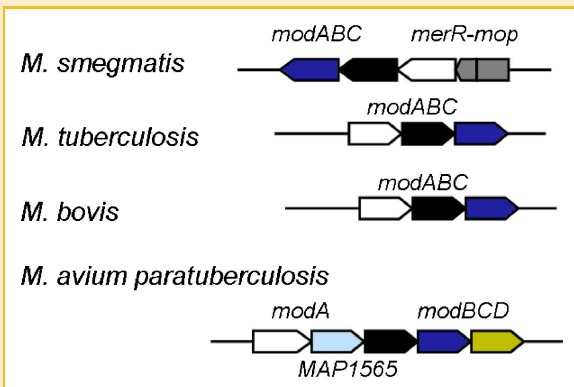
Fig. 4. Genomic organization of MoaABC in mycobacteria. Different genes are show by the indicated color schemes. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

organisms lack both ModE and its variants suggests that alternative regulators are present in these organisms for ModABC regulation [Zhang and Gladyshev, 2008]. Probably there are also alternative regulators to regulate the transport of Mo in mycobacteria.

In *E. coli*, all genes involving in Moco biosynthesis organize five operons including *moa*, *mob*, *mod*, *moe*, and *mog*. Except *mob* [Iobbi-Nivol et al., 1995] and *mog* [Vergnes et al., 2004] are expressed constitutively, the transcription of *moa* [Spiro and Guest, 1990; Anderson et al., 2000], *mod* [Miller et al., 1987; Grunden and Shanmugam, 1997], and *moe* [Hasona et al., 2001] are regulated by several factors such as Mo, oxygen, and so forth. Due to the aberrant construction of genes in mycobacterial genomes, the expression and regulation of these genes are complicate and elusive.

CONCLUSION

Mo enzymes have a significant role in physiological function of mycobacteria. The common cofactor of Mo enzymes is an interesting research area. Not only the biosynthesis of Moco is complex, but also the delivery of the mature cofactor onto the apoenzymes is sophisticated. For example, the maturation of *E. coli* nitrate reductase A involves in the incorporation of the Moco to the apoprotein, the NarJ chaperone is required [Blasco et al., 1998; Lanciano et al., 2007] and the complex composed of MogA, MoeA, MobA, and MobB is in charge with the delivery of the mature Moco onto the aponitrate reductase A [Vergnes et al., 2004]. More profound exploration of the mechanism of cofactor biosynthesis, cofactor allocation, and the assembly of Mo enzymes might open new avenues to design new drug against tuberculosis.

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