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Dissertation for the degree of Doctor of Philosophy

AccD6 protein as the functional carboxyltransferase in mycolic acid biosynthesis of the slow- and fast-growing mycobacteria

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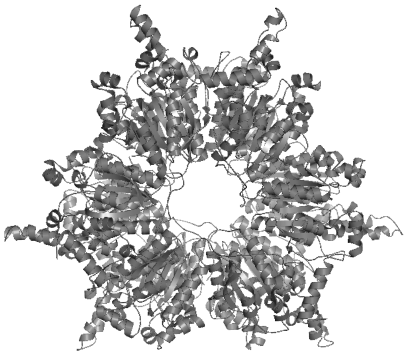

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SUMMARY

Malonyl-CoA is the universal, two-carbon substrate for the synthesis of mycolic and other fatty acids in mycobacteria. It is generated by the carboxylation of acetyl-CoA in a biotin-dependent, two-step reaction catalyzed by acetyl-CoA carboxylase (ACC) and incorporated into the growing acyl chain during the repetitive cycle of the fatty acid synthase I and II (FAS I/FAS II) reactions.

Each half-reaction is catalyzed by a specific ACC subunit: the first step by biotin carboxylase (BC; subunit α) and the second step by carboxyltransferase (CT; subunit β), each catalytic subunit being encoded by a separate gene. Since the β subunits confer the substrate specificity of ACC, characteristics of the mycobacterial carboxylases and encoding genes seems to be the key issue in understanding the malonyl-CoA synthesis as well as other precursors in *M. tuberculosis* lipid biogenesis.

The main objective of the study was confirmation of AccD6 protein function as the component of malonyl producing acetyl carboxylase in *Mycobacterium*.

By construction and lipid analysis of *M. tuberculosis* mutants we have demonstrated that *accD6* (Rv2247) encodes the essential carboxyltransferase, responsible for synthesis of the malonyl-CoA in tubercle bacillus *in vivo*. Strikingly, our study revealed that although *accD6* homologue of *M. smegmatis* (MSMEG_4329) possesses the same essential function, it is dispensable. The deletion of *accD6* in *M. smegmatis* did not affect the cell envelope integrity.

To identify alternative CT subunit of ACC in this species, we tested the essentiality and possible involvement in mycolic acid biosynthesis of all CT genes in *M. smegmatis*. Analysis allowed the identification of MSMEG_6391 and MSMEG_1813 (*M. tuberculosis accD4* and *accD5* homologues) as the only essential CT genes in *M. smegmatis*. The study on the function of both genes led us to the conclusion that protein encoded by MSMEG_1813 is bifunctional, carrying propionyl but also acetyl carboxyltransferase activity that allow replacing the MSMEG_4329 (*accD6*) function in this species.

Since the analysis performed on *M. tuberculosis* showed that the AccD5 in this species is unable to replace the function of acetyl-CoA carboxyltransferase, we have

demonstrated that the observed difference in *accD6* essentiality lays in the different substrate specificity of AccD5 protein between analyzed mycobacterial species.

In addition, although in both species *accD6* is a member of the FAS -II transcriptional unit and its expression is controlled by the P_{fasII} promoter, it was found that *accD6* of *M. tuberculosis,* but not *M. smegmatis,* possesses its own, additional promoter (P_{acc}). This implies that in the pathogenic strain, *accD6* expression may be controlled by two regulatory sequences, P_{fasII} and P_{acc}. Although the additional promoter seems not to participate in supporting the physiological expression level of $accD6_{Mtb}$ under standard growth conditions, it is able to sustain the expression of this gene on a level allowing for cell survival in the absence of P_{fasII}.

Presented results provided useful data concerning probable involvement of all AccD proteins in mycolic acid biosynthesis. Moreover the key role of AccD6 in *M. tuberculosis* as the donor of malonyl-CoA in mycolic acid biosynthesis was confirmed. Finally, it is also the first step to identify effective method for inhibition of tubercle bacillus lipid biosynthesis by direct blocking of the AccD6 protein activity.