The Actinobacterial *mce* Operon: Structure and Functions

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Abstract—The results of studies on *mce* operons of the causative agent *Mycobacterium tuberculosis* and actinobacteria are summarized. Mce transporters belong to a group of cell-wall proteins. The genome of *M. tuberculosis* contains four *mce* operons that encode complexes, each of which consists of two integral membrane proteins (YrbEB) followed by six other Mce proteins (MceA−F). These proteins are functionally similar to ABC transporters. Despite their significant role in the pathogenesis of *M. tuberculosis* infection, Mce proteins remain poorly studied due to their complex structure and operon regulation. However, a number of their functions have been characterized; they include the penetration of host cells by *M. tuberculosis* and its survival, as well as the transport of cholesterol and mycolic acids, which are pathogenicity factors of no small importance. The expression of *mce* operons depends on many factors, such as the growth phase, the culture medium, and the localization of *M. tuberculosis* infection. Today, strains of *M. tuberculosis* and *M. bovis* with deleted *mce* operons are considered candidates for the development of new attenuated vaccines that might one day replace the *M. bovis* BCG vaccine, which is no longer deemed strong enough to hold back the tuberculosis epidemic.

Keywords: virulence *Mycobacterium tuberculosis*, *mce* operon, actinobacteria, ABC transporters

INTRODUCTION

Tuberculosis is currently one of the most common infectious diseases and one of the ten leading causes of death in the world (*Global...*, 2019). Of particular danger is the increase in the proportion of strains with multidrug and wide drug resistance and increased virulence (Ribeiro et al., 2014; Lange et al., 2018). Virulence refers to the ability of a pathogen to infect a host organism. It is determined by a number of factors and depends both on the host, including its immunoresponse, and on the pathogen itself (Marimani et al., 2018). The clinical manifestations and epidemiology of tuberculosis are thus determined by the balance between the host immune system and pathogen virulence systems. The genome of *Mycobacterium tuberculosis* includes more than 300 genes that encode various virulence factors involved at different stages of the colonization of the host organism, including adhesion, cellular incorporation, avoidance of the immune system response, etc. (Forrellad et al., 2013; Prozorov et al., 2014). These include genes that determine the biosynthesis of mycolic acids, translational regulators of WhiB, serine-threonine protein kinases, genes encoding type-VII secretion systems, genes of toxin-antitoxin systems, etc. Their products are involved at various stages of the infection process and make it possible to colonize the mucous membrane of the host, to invade cells, to avoid the immune system response, to survive adverse conditions, etc. These pathogenicity factors include Mse proteins (Mammalian cell entry). A piece of DNA *M. tuberculosis* 450 bp H37Ra, which gives *Escherichia coli* cells the ability to penetrate and survive in mammalian cells, was first identified in 1993 (Arruda et al., 1993). It is with this function, one of the first known, that the name of these proteins is associated. They are among the proteins of the cell wall and are involved in the penetration of *M. tuberculosis* cells in macrophages and their survival, as well as in the transport of cholesterol and mycolic acids. At present, Mce proteins have demonstrated presence of antigenic activity, which is of interest for their use as a potential basis for a genetically engineered vaccine (Ahmad et al., 2004).

SPREAD OF *mce* OPERONS IN BACTERIA

These proteins are not specific to *M. tuberculosis* and Mycobacterium tuberculosis complex. They are found in various pathogenic and nonpathogenic mycobacteria, such as *M. intracellulare, M. smegmatis,* and *M. scrofulaceum,* (Parker et al., 1995). In addition to mycobacteria, Mce operons are noted in the genomes of many free living and pathogenic microorganisms, including the families Intrasporangiaceae, and Streptomycetaceae, Nocardioidaceae (Casali and Riley, 2007; Mohn et al., 2008; Nakayama and Zhang-Akiyama, 2016; Hemati et al., 2019).

According to Cole et al. (1998), *mce* modules are the result of tandem duplication; therefore, a number of mycobacteria have several *mce* copies. Thus, *N. farcinica* have two copies: *mce7* and *mce8*. In general, there is a tendency toward an increase in the number of *mce* operons in fast-growing mycobacteria as compared to slow-growing ones (Miller et al., 2004). *M. smegmatis* contains six *mce* operons, *mce1–7*. In this case, this microorganism has no *mce3* operon; the *mce1* operon is similar to that of *M. tuberculosis*, but it includes two additional genes (*MSMEG_5902* and *MSMEG_5893*), while there is an insert in operon *mce5* between genes *mce5A* and *mce5B* (García-Fernández, 2017). A characteristic feature of operon *mce3* is its decrease in a number of mycobacteria, such as *M. bovis* BCG, *M. smegmatis, M. microti,* and *M. leprae* (Ahmad et al., 2004). The latter is characterized by the presence of only one *mce* module in the genome. The presence of *mce* operons in nonpathogenic bacteria may indicate the existence of functions other than virulence in them. In particular, the participation of *mce* operons in cholesterol catabolism can explain their presence in free-living saprophytic bacteria, since there are steroid substances found in nature that bacteria can use as substrates for growth. At the same time, it was established that *mce* operons and their analogs, which are found in the genomes of certain pathogenic bacteria, participate in virulence. In *Leptospira interrogans,* the Mce protein participates in adhesion, allowing interaction with the receptors of the host cells (Cosate et al., 2016). Expression of *gltT* gene, an analog of the *mce* operon of *Neisseria meningitidis*, results in strains with high invasiveness and hypervirulence (Pagliarulo et al., 2004).

MAIN STRUCTURAL AND FUNCTIONAL FEATURES OF Mce PROTEINS
in *Mycobacterium tuberculosis*

Proteins of Mce actinobacteria, including *M. tuberculosis* are ATP-binding cassette transporters. YrbE proteins are functionally homologous to permeases and have a region in the penultimate cytoplasmic loop that can serve as a site of interaction with ATPases, while other Mce are homologous to substrate-binding proteins (Casali and Riley, 2007). Between them, *mce* operons have a certain homology and are apparently the result of tandem duplication (Cole et al., 1998). All Mce proteins are characterized by a conserved TIGR00996 domain (IPR005693). It includes 304 amino acids, which is typical for actinomycetes.

Some of the *mce* operons in actinomycetes include the *mkl* gene, which encodes an ATPase similar to ATPases of ABC transporters. A region in the cytoplasmic loop of YrbE that can serve as a site of interaction with Mkl-ATPase was identified. Mkl was found only in those actinomycetes with a genome containing genes that encode YrbE and Mce proteins

Operons often also include other genes that are functionally associated with *mce* or are involved in their regulation. Thus, the composition of *mce1* currently includes *fadD5* (Rv0166), the intended function of which is to participate in the first stage of the degradation of fatty acids (Dunphy et al., 2010). In addition, before the operons *mce1–3* located transcriptional regulators *mceR*.

mce1 Operon

The *mce1* operon is present in all types of mycobacteria. The operon contains six genes (*mce1A–mce1F*) that encode transporter proteins localized in the cell wall. (Chitale et al., 2001; Shimono et al., 2003; Stavrum et al., 2012). This operon is the most conservative of all *mce* operons and is the only one found in *M. leprae*, which is characterized by a strongly reduced gene (Forrellad et al., 2014). The *mce1* operon has been studied for quite some time, but the results of these studies are often contradictory. Thus, the *M. tuberculosis* strains with knockout for *mce1* demonstrate a hypervirulent phenotype when administered intravenously or intraperitoneally, while their virulence, conversely, is reduced upon intratracheal administration (Shimono et al., 2003; Gioffré et al., 2005).

The functions of the Mce1A protein were studied in greatest detail. This protein promotes the penetration of macrophages by *E. coli* cells and colloidal gold particles (Chitale et al., 2001). The functional domain of Inv3, which plays a key role in this, is a sequence comprising 22 amino acids (Lu et al., 2006).

Its functions are supposed to be in the transport of palmitic acid and mycolic acids, which are important virulence factors involved in the protection of the pathogen from the host immune system by inhibiting the inflammatory response in macrophages. Deletion of this operon leads to their accumulation in the extracellular space. The lipid profile of the cell also undergoes serious changes, including a decrease in the level of saccharolipids and glycerophospholipids and an increase in the level of alpha, methoxy, and ketomycolic acids. This is due to the fact that the deletion of the *mce1* operon leads to a decrease in the expression of genes involved in the transport and metabolism of lipids *mmpL8, mmpL10, stf0, pks2* and *papA2,* the products of which are involved in the anti-inflammatory response. At the same time, the transcription of *mmpL3, fasI, kasA, kasB, acpM,* and *RV3451,* which are involved in the increase in the transport and metabolism of mycolic acids. Thus, deletion of the *mce1* operon, which changes the metabolism and composition of cell-wall lipids, affects the nature of the host's immunoresponse and determines the nature of the infection (Queiroz et al., 2015). In addition, this operon is expressed at the initial stage of infection, and its products are involved in the transition from the fast- to slow-growth phase (Beste et al., 2009).

mce2 Operon

The *mce2* operon is present in all mycobacteria of the tuberculosis complex and is typical of the structure of *mce* operons and demonstrates significant homologous similarity with the *mce1* operon (Ahmad et al., 2005). A feature of *mce2* is the presence of a gene fragment *Rv0590A* between *mce2B* and *mce2C* (Zhang and Xie, 2015). Mce2 proteins are thought to be involved in the metabolism and import of sulfolipids (Marjanovic et al., 2011). Deletion of the *mce2* operon in the strain *M. tuberculosis* H37Rv does not affect growth in vitro, but causes a weakening of the strain when laboratory animals are infected (Marjanovic et al., 2011). Deletion of this operon, as well as the gene *phoP,* in *Mycobacterium bovis* leads to a loss of virulence and the formation of an attenuated strain (García et al., 2016). The functions of individual operon proteins have not been studied to date, with the exception of Mce2E, which suppresses the innate immunoresponse of macrophages and promotes the proliferation of epithelial cells. Mce2E inhibits the activation of signaling pathways regulated by extracellular signal kinase (ERK) and Jun N-terminal kinase (JNK), which, in turn, is activated by mitogen-activated protein kinase (MAPK), which leads to a decrease in the expression of TNF and IL-6 in macrophages (Uchiya et al., 2013). In addition, Mce2E stimulates the cellular proliferation of epithelial pulmonary cells. Thus, Mce2E is a new, multifunctional virulence factor that regulates the host's immunoresponse (Qiang et al., 2016).

mce3 Operon

As previously mentioned, the *mce3* operon is absent in a number of mycobacteria, such as, *M. bovis* BCG, *M. smegmatis, M. microti,* and *M. leprae* (Ahmad et al., 2004, Zhang and Xie, 2011). It was established with the HeLa model cell line and latex beads that the Mce3A and Mce3E proteins that make up the operon are involved in invasion processes (El-Shazly et al., 2007). An immunogenic reaction was shown for the proteins Mce3A, Mce3D, and Mce3E (Ahmad et al., 2004, Zhang and Xie, 2011). The expression of the *mce3* operon is downregulated by *mce3R,* which belongs to the tetR family. It, in turn, is under the regulation of its own product, Mce3R (Santangelo et al., 2008). These two genes are involved in phosphatidylinositol biosynthesis and lipid degradation, respectively (Santangelo et al., 2008).

It was shown that Mce3C participates in the adhesion to and penetration of macrophages, in which the Arg-Gly-Asp motif (RGD) is involved. Mce3C interacts with the β2 integrins of the host, allowing penetration of the cell. Integrin binding to Mce3C stimulates β2 integrin-dependent signal adapters and induces local actin rearrangement at the site of mycobacterial invasion (Zhang et al., 2018).

It was determined that the *mce3* operon is expressed in the cells of the pathogen exclusively upon infection of the lung tissue; no expression was found when the pathogen penetrated spleen cells. Thus, the expression of the *mce3* operon is tissue-specific (Kumar et al., 2003).

Mutations were detected in the genes of the *mce3* operon of *M. tuberculosis.* A thymine-to-cytosine mutation at position 44 in the *mce3B* gene is characteristic of isolates of the highly virulent F15/LAM4/KZN line, which is characterized by a high level of drug resistance. Another mutation in which adenine is replaced by cytosine at position 1229 of *mce3F* is characteristic of some isolates of the subline B0/W-148, which also has a high level of drug resistance and increased virulence. The subregion is named B0/N-90 (Mikheecheva et al., 2017).

mce4 Operon

The *mce4* operon is expressed in the stationary phase of mycobacterial growth culture in vitro or in the cells of laboratory animals. The degree of homology of this operon is quite high among various mycobacteria (Mitra et al., 2005; Saini et al., 2008; Timms et al., 2015).

As with Mce1A, the Mce4A protein gives *E. coli* cells the ability to penetrate HeLa cells (Zhang and Xie, 2011). Deletion of the *mce4* operon leads to a significant reduction in virulence *Mycobacterium tuberculosis* (Zhang and Xie, 2011; Khan et al., 2016). For Mce4F, participation in virulence (i.e., invasion) was predicted via analysis *in silico* (Rodríguez et al., 2015). However, the main function of these proteins is to participate in the catabolism of cholesterol. This is part of the membranes of the host cells, which mycobacteria are used as a source for their own development.

The Mce4E protein inhibits macrophage activity, disrupts phagocytosis, and thus plays an important role in the interaction of the host with the pathogen, since alveolar macrophages have an antigen-presenting function and are involved in the production of cytokines and TNF-α (Xu et al., 2008).

Thus, Mce proteins are involved in the development of the infectious process via inhibition of the activity of alveolar macrophages and the host immune system interactions, and they participate in the formation of granulomas (Marjanovic et al., 2010). Being functionally and structurally similar to ABC transporters, they are involved in the transport of lipids, which are virulence factors (Hemati et al., 2019). *Mycobacterium tuberculosis* Mce proteins of can help pierce the outer lipid layer and can form a channel through this lipid bilayer.

In, *Mycobacterium avium* subsp. *paratuberculosis,* Mce proteins are a key factor in the interaction with the host immune system (Ghosh et al., 2012).

FEATURES OF THE EXPRESSION OF *mce* OPERONS AT DIFFERENT STAGES OF GROWTH

In the study of the expression of these operons, it was initially found that *mce1* expression is observed at the exponential stage of culture growth, which almost disappears in the stationary phase when *mce4* begins to be expressed. The *mce2* and *mce3* operons were expressed exclusively in the tissues of infected animals in the stationary phase (Kumar et al., 2003). It was subsequently found in 2016 that *mce1* continues to be expressed in the stationary phase (Singh et al., 2016). Obviously, the difference from the results of Kumar et al. (2003) are methodological differences (Kumar et al., 2003; Singh et al., 2016). The expression of the *mce* operon occurs mainly in the stationary growth phase, in conditions of depleted nutrients and the accumulation of metabolic products. This can be interpreted as the participation of *mce* in survival in adverse conditions, including macrophages. This is also indicated by the fact that the expression of genes such as *Rv3843c*, *ponA1*, *uvrA*, *Rv3070*, *pks2*, *ctpV*, *prrA,* and *sigE* is observed in the stationary growth phase, which is characteristic of the development of *M. tuberculosis* in vivo in the tissues of infected laboratory animals (Singh et al., 2016).

CONCLUSIONS

The *mce* operons of *M. tuberculosis* undoubtedly play an extremely important role in pathogenesis and virulence. Their functions are very diverse and consist of the transport of other virulence factors, including mycolic acids and lipids, and direct interaction with the host cells and its immune systems. Although this group of genes has been studied for two decades, their functions and role in the pathogenesis of *M. tuberculosis* are not completely clear. The expression of *mce* operons depends on the growth stage, the used model systems (in vitro or in vivo), and the localization of the infectious process in the laboratory animals. In addition, the presence of single-nucleotide polymorphisms, which gradually appeared and were accumulated during evolution, is characteristic of *mce* genes. A significant part of the polymorphisms leading to the replacement of the amino acid can affect the structure of the protein product, changing its properties, including the antigenic activity (Ahmad et al., 2004; Mikheecheva et al., 2017). Analysis of *in silico* mutations in the genes of the *mce1* and *mce4* operons in *M. tuberculosis* made it possible for some of them to predict a significant effect on the structure and antigenic properties (Pasricha et al., 2011). These genes have no analogs in the human genome, which makes them ideal candidates for vaccine development. In addition, the *mce* operon is absent in *M. bovis*; therefore, prior BCG vaccination will not affect the immune response.

In this regard, this group of genes is of undoubted interest for research and could be used create new genetically engineered vaccines.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

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