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LETTERS TO THE EDITOR

Expression of Small RNAs of *Mycobacterium tuberculosis* in Murine Models of Tuberculosis Infection

D. V. Ignatov[a], O. Yu. Timoshina[a], N. N. Logunova[b], T. A. Skvortsov[a], and T. L. Azhikina[a, 1]

[a] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, 117997 Russia*

[b] *Central Institute for Tuberculosis, Russian Academy of Medical Sciences, Yauzskaya al. 2, Moscow, Russia*

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Abstract—The determination of the mechanisms contributing to the survival of pathogenic bacteria in the infected organism and the possible ways of their blocking is a promising approach to the development of new methods of affecting these bacteria. Among these mechanisms, the regulation of bacterial metabolism by small RNAs attracts particular interest since it has been found recently to play an important role in the bacterial pathogenesis. We have studied the expression of three most highly expressed small RNAs of *Mycobacterium tuberculosis*: MTS0997, MTS1338, and MTS2823 during tuberculosis progression in the strains of mice having different genetic resistance to the disease. It has been shown that the maximum expression of these small RNAs occurs at earlier stages of infection.

Keywords: Mycobacterium tuberculosis, small RNAs, expression in vivo

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INTRODUCTION

In the last decade, a great number of small regulatory transcripts have been identified in bacteria, and their involvement in some processes has been established. Thus, it was found that most small RNAs encoded in intergenic regions are synthesized in response to the impact of external factors. This helps bacteria to adapt to varying environmental conditions, e. g., in the case of the adaptation of pathogens to the host organism. It is also known that some non-coding RNAs control the expression of genes encoding the virulence factors in some bacteria [1].

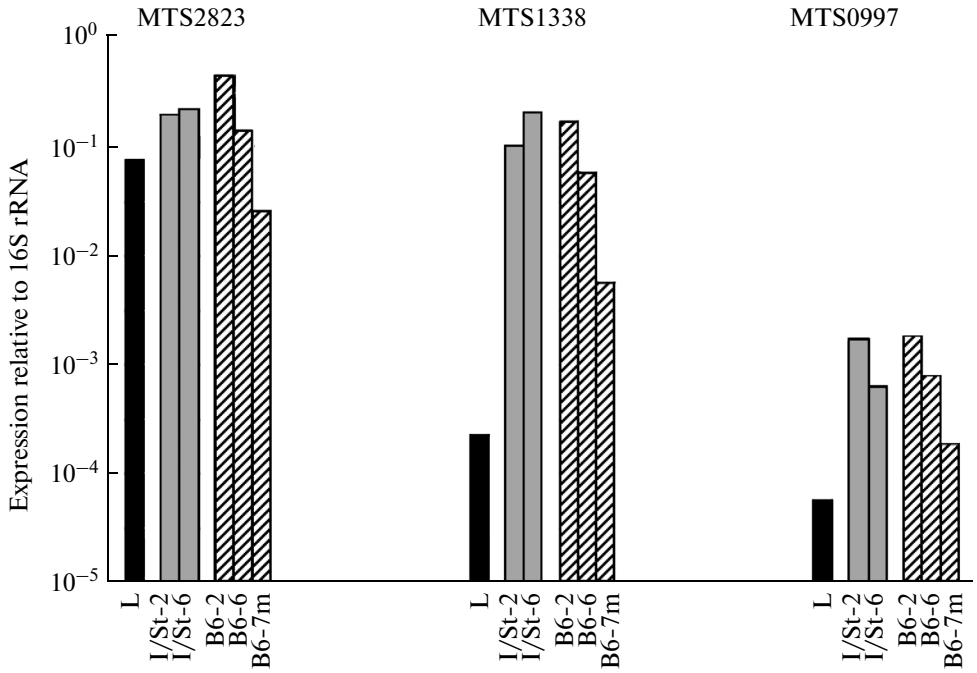
Small non-coding RNAs were detected in many bacterial species, among them, *Mycobacterium tuberculosis* [2, 3]. According to the data of the massive sequencing of the *M. tuberculosis* transcriptome, small non-coding RNAs amount to 58% of the fraction of total RNA purified from rRNA.

The goal of the present work was to study the dynamics of the expression of small RNAs of *M. tuberculosis* during the disease progression in vivo. We chose three small RNAs: MTS0997, MTS1338, and MTS2823; it has been shown earlier that the level of their expression in the lungs of infected mice increases at late stages of chronic tuberculosis (nine months after the infection) [4]. It was of interest to determine whether the stage of the disease and the genetic features of the host organism affect the level of expression of these small RNAs. For this purpose, we used the models of tuberculosis infection in mouse strains with different genetic resistance to tuberculosis [5].

The aerogenic infection with low doses of *M. tuberculosis* results in chronic but readily controlled infection in resistant animals of strain B6 and fatal lung pathology in susceptible animals of strain I/St. Mice of the I/St strain die from progressing tuberculosis 120—150 days after the infection, and B6 mice survive for eight to ten months. The difference in the lung CFU counts between I/St and B6 mice reaches two orders of magnitude within the first month after the infection and then remains at this level throughout the observation [5]. To estimate changes in the expression of small RNAs during the disease progression, we chose the following time points: 2 weeks after the infection (the CFU counts in both strains is the same; the immune response is not yet developed; possible differences in the bacterial transcriptome are minimal); 6 weeks after the infection (substantial difference in the CFU counts; the bacterial transcriptome reflects bacterial adaptation to the genetic features of the host); and 7 months after the infection (only B6 mice survive; the stage of chronic infection). For each of these time points, total RNA samples were obtained: I/St-2, B6-2, I/St-6, B6-6, and B6-7m;

Abbreviations: CFU, colony-forming unit; RT-PCR, reverse transcription polymerase chain reaction.

[1] Corresponding author: phone: +7 (495) 330-65-47; fax: +7 (495) 330-65-38; e-mail: tatazhik@ibch.ru.



RNA	L	I/St-2	I/St-6	B6-2	B6-6	B6-7m
MTS2328	$(7.70 \pm 3.01) \times 10^{-2}$	$(1.99 \pm 0.13) \times 10^{-1}$	$(2.22 \pm 0.03) \times 10^{-1}$	$(4.58 \pm 0.44) \times 10^{-1}$	$(1.41 \pm 0.03) \times 10^{-1}$	$(2.54 \pm 0.14) \times 10^{-2}$
MTS1338	$(2.26 \pm 0.07) \times 10^{-4}$	$(1.05 \pm 0.14) \times 10^{-1}$	$(2.08 \pm 0.05) \times 10^{-1}$	$(1.72 \pm 0.19) \times 10^{-1}$	$(5.84 \pm 0.12) \times 10^{-2}$	$(5.55 \pm 0.24) \times 10^{-3}$
MTS0997	$(5.75 \pm 0.09) \times 10^{-5}$	$(1.69 \pm 0.32) \times 10^{-3}$	$(6.28 \pm 0.39) \times 10^{-4}$	$(1.78 \pm 0.08) \times 10^{-3}$	$(7.76 \pm 0.05) \times 10^{-4}$	$(1.83 \pm 0.10) \times 10^{-4}$

Expression of small RNAs: MTS0997, MTS1338, and MTS2823 in murine models of tuberculosis infection B6 (a resistant strain) and I/St (a susceptible strain) at different times after the infection and in the logarithmic growth phase of *M. tuberculosis* culture (L). Figures in the designations of total RNA samples correspond to the time after the infection of mice: "2", two weeks; "6", six weeks; and "7m", seven months. The ordinate axis is given in the logarithmic scale. In the table under the diagram, the averaged data of three separate experiments and the standard error of means are given.

total RNA from *M. tuberculosis* culture in the logarithmic growth phase (L) was used as a control (figure).

RNA was isolated by homogenizing the murine lungs in the Trisol reagent (Invitrogen), and further procedures were carried out according to the standard protocol using phenol and chloroform. cDNA was synthesized with random primers and Mint reverse transcriptase according to manufacturer's instructions (Evrogen, Russia). The expression of small RNAs was estimated by real-time RT PCR. The sequences of oligonucleotide primers are given in the table.

The figure shows the expression level of MTS0997, MTS1338, and MTS2823 relative to 16S rRNA at different times after the infection in mice with different genetic resistance to tuberculosis and in the logarithmic culture. A high level of the expression of the three small RNAs is observed even within two weeks after the infection. The number of MTS2823 transcripts in infected lungs of the mice of both strains is one order of magnitude greater than that in the cell culture. The number of MTS0997 transcripts in vivo is more than 100 times higher than in culture. The greatest difference between the expression under in vivo and in vitro conditions is observed for MTS1338. The numbers of transcripts differ by three orders of magnitude. These changes in the expression may indicate that MTS1338 and MTS0997 are to a much greater extent important for the persistence of mycobacteria in macrophages than MTS2823. This is also evidenced by the fact that the level of the MTS1338 and MTS0997 expression in chronic infection (seven months after infection) remains higher than in the culture, whereas the level of MTS2823 expression in chronic infection drops below that in the culture.

MTS2823 is a rather long (~300 nt) transcript, which is transcribed from the region of the DNA plus strand between the genes Rv3661 and Rv3662c. It was shown that the enhanced expression of MTS2823 leads to significant changes in the expression of the

Oligonucleotide primers used for real-time RT-PCR

Name	Sequence (5' → 3')
qPCR_MTS1338_for	GGGGAAACCCGGTGATCTG
qPCR_MTS1338_rev	GGTAGGTCAAACCGGGTGTACAT
qPCR_MTS2823_for	AAGCCCGGTGAGGCCAA
qPCR_MTS2823_rev	CGTCGATGCCATCTGCTGTT
qPCR_MTS0997_for	GAAGCAGGCCCGGTTAGTGA
qPCR_MTS0997_rev	GGCAGACCCGGCGTGACT
16S_for	TACGTAGGGTGCGAGCGTTG
16S_rev	CCCGCACGCTCACAGTTAAG

components of the methyl-citrate cycle [4], which is one of the most important carbon sources for cell growth [6]. The mechanisms of regulation with the involvement of MTS2823 remain so far unknown; however, the constantly high level of the expression of this RNA under different conditions in vitro and in vivo may indicate that it plays a structural role.

At the same time, according to the data reported in [4], the small RNA MTS1338 is little synthesized in the logarithmic growth phase, but accumulates in rather great amounts in the stationary phase. The expression of MTS1338 is controlled by the factor DosR, which affects the adaptation of bacteria to the anaerobic environment, including latent infection. This may account for the abrupt increase in its expression in vivo as well as for the fact that the level of its expression even in the chronic state is higher than in the culture.

Another interesting fact is a fall in the expression of all three RNAs during the disease progression in mice of the tuberculosis-resistant strain B6 at the time points: 2 weeks, 6 weeks, and 7 months. The character of the fall is similar for all three RNAs, which can be due to a decrease in the total amount of transcripts in the chronic stage [7, 8].

We expected to see differences in the expression of small RNAs in infected organisms with different genetic resistance to tuberculosis since, even by week 6 after the infection, they develop different immune responses [5]; however, these differences were insignificant. On the whole, it can be noted that the type of the immune response of the host is not the main factor that determines the expression of these small RNAs; however, further studies are needed to confirm this suggestion.

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