

# Mechanism of Manganese Transport Regulation in *Brucella* Involving a Long RNA Helix

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**Abstract**—It has been found that divalent cation transporters of the Nramp family in eukaryotic cell phagosomes and bacteria that parasitize in these cells compete for metals, which is significant for bacterial survival. Long helices were determined by means of our algorithm in the 5'-untranslated region for each mRNA in *Brucella*. Long helices of quite similar nucleotide composition were found in mRNAs that encode manganese transporters and Ni-dependent glyoxalase I. We suggest that long helices in these regions are involved in the regulation of RNA stability.

**Key words:** *Brucella*, manganese transport, RNA helix

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## INTRODUCTION

$\alpha$ -Proteobacteria of genus *Brucella* are intracellular parasites of sheep (*B. melitensis*), cows (*B. abortus*), swine (*B. suis*) [1]. Humans are most susceptible to the species *B. melitensis*. The work proposes a genus *Brucella*-specific new mechanism of regulation of expression of some genes, including genes of the Nramp family and metalloprotein genes. The Nramp family unites transporters of divalent metal cations, in particular manganese  $Mn^{2+}$ , iron  $Fe^{2+}$ , zinc  $Zn^{2+}$ , and copper  $Cu^{2+}$ . Transporters of the Nramp family have been found both on the macrophage phagosome membrane in mammals [2, 3] and in bacteria [4]. These transporters play an important role during phagocytosis and also during bacterial survival in the course of incomplete phagocytosis, for example, *Brucella* are intracellular parasites. The transporters of the bacterium and the macrophage compete with each other, favoring survival of the bacterium inside the macrophage [2–4]. The mentioned metals enter into the composition of many enzymes protecting bacteria against oxidative stress inside the phagosome. A suggestion has been put forward that though transporters of the Nramp family usually deliver cations into the cells, they also remove from the cell some cations that are present there in excess [5].

For *Mycobacterium tuberculosis* it has been experimentally shown that the concentration of mRNA corresponding to a transporter of the Nramp family is regulated by the concentration of cations. For this case the possibility of transport of different cations by one and

the same transporter of this family has been demonstrated, and elevation of the concentration of some cations can lower the efficiency of transport of other cations [4].

In our work a new mechanism of regulation is proposed, based on degradation of mRNA with participation of long RNA helices positioned before or after the regulated gene. Some ribonucleases are metalloproteins including manganese. Manganese is necessary also for ribonuclease H II causing degradation of RNA in the composition of an RNA–DNA hybrid. For prediction of RNA helices, use was made of the algorithm proposed by the authors. Genomes of bacteria were obtained from the GenBank database, ftp://ftp.ncbi.nlm.nih.gov. The names of orthologous genes from genus *Brucella* and from *E. coli* are presented in the table.

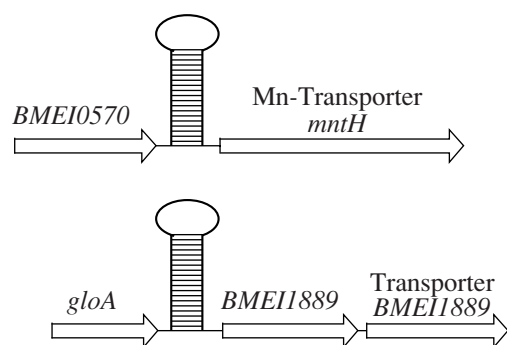
## RESULTS

With the use of our algorithm of searching for RNA helices in the gene leader regions, genes were discovered in front of which there are long RNA helices with a periodic repeat of consecutively eight nucleotides in the helix arms, sometimes with continuation of the repeat into the helix loop (see Fig. 1). In each case these helices were found between co-directed genes positioned at a small distance from each other (up to 300 nucleotides) and without the usual terminator between them. Therefore it is not excluded that they enter one operon. The results obtained with the help of the algorithm proposed by us pertain to the following two cases (Fig. 2).

**The first case** consists in that before the long helix there is a hypothetical gene *BME10570* and after it—

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**Fig. 2.** Mutual positioning of coding regions and the long hairpin for two mRNAs in *B. melitensis* 16M. For other brucellae the positioning of the hairpin and coding regions is analogous. At the top, situated before the long helix is the coding region of hypothetical gene *BMEI0570* and after it—the coding region of gene *mntH* from family Nramp (Pfam, PF01566). At the bottom, situated before the long helix is the coding region of gene *gloA* for Ni-dependent glyoxalase I (EC 4.4.1.5) and after it—gene *BMEI1889* of a hypothetical protein. Further after this gene follows the gene *BMEI1890* of a hypothetical transporter with unknown function, including domains DUF1775 and DUF461, the second of which is characteristic of many transporters.

*B. melitensis* biovar Abortus 2308 instead of a quadruple repeat we found a double repeat with the same period.

Sequences 100–150 nucleotides long homologous to fragments of the helices we found or their loops are found also in other places of *Brucella* genomes, however, they do not enter into the composition of any long RNA helix. But such sequences are not found in other bacteria. By analogy to well-known cases one can think that the indicated helices in *Brucella* emerged from transposable IS elements ([6] pp. 230–232).

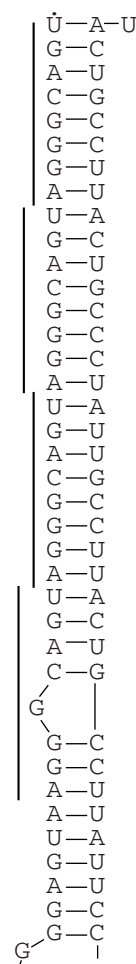
We suppose that the helices found by us are transcribed together with the indicated genes and form on mRNA rather than on DNA for the reason that they contain GU pairs. In this case a long helix can form only on RNA. Besides that, these helices are situated between genes of one DNA strand, which are positioned at a small distance from each other, i.e. they can enter a unified operon. The conservativeness of the helix—long hairpin with a repeat in its arm of literally one word AGGCAGU allows a suggestion that it is under pressure of stabilizing selection and consequently exists as a helix on mRNA. Though in many bacteria the transcription of gene *mntH* is regulated by protein MntR binding DNA, this protein is absent in *Brucella*. The presence of a conservative long helix on mRNA that is absent (except *Brucella*) in all other species presented in GenBank allows a suggestion that *Brucella* possesses another regulation of gene *mntH*. This supplements the statement from [7] where it is supposed that gene *mntH* belongs to the MUR regulon.

**The hypothesis** consists in that the considered helix with the indicated repeat of nucleotides serves as a place for binding of a metalloprotein including manga-

nese or zinc with mRNA, which influences the mRNA stability. Metalloprotein binding and consequently mRNA stability depends on the concentration of metal cations.

The candidate for the role of the corresponding regulatory metalloprotein can be ribonuclease H II (EC 3.1.26.4), which binds manganese and causes mRNA degradation at the expense of ribonuclease binding with the long helix. In the presence of a sufficient amount of manganese the long RNA helix found by us is subjected to degradation by this ribonuclease. If there is little manganese, then the ribonuclease does not destroy mRNA containing the transporter gene *mntH*, and manganese goes into the bacterium.

The discovered RNA helices with periodic structure can also serve as a place for cooperative binding of a regulatory protein protecting mRNA from degradation in the case if there is little cations. Therewith the regulatory element can be situated both at the 5'-leading and the 3'-trailing region of the regulated gene. A similar case is given, for example, in work [6].



**Fig. 3.** Stalk of the long hairpin before gene *mntH* in *B. melitensis* 16M. The stalk contains the repeat marked by the line.

Though the closest homologs of the MntH transporter from *Brucella* are specific to manganese, by analogy to *Mycobacterium* it can be suggested that MntH in *Brucella* can take part in the transport of several divalent metals. Long hairpins with a repeat of one and the same word in the arm are found both before the MntH transporter and before a transporter of unknown specificity. Therefore it can be suggested that this second transporter following the gene of Ni-dependent glyoxalase I and entering together with it a unified operon is also associated with transport of divalent metal cations.

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