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STRUCTURAL AND FUNCTIONAL ANALYSIS

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C-Terminus of Transcription Factor TnrA from *Bacillus subtilis* Controls DNA-Binding Domain Activity but Is Not Required for Dimerization[1]

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Abstract—The transcription factor TnrA, which belongs to the MerR transcription regulators, in *Bacillus subtilis* controls genes of nitrogen metabolism during nitrogen limiting conditions. As all the DNA-binding proteins, it is active as a dimer in cells, but the dimerization site is still unknown. The multiple alignment of TnrA homologs from other *Bacilli* allowed to identify the putative dimerization sites. Using the C-terminal truncated TnrA proteins it is established, that, in contrast to other MerR-proteins, the TnrA C-terminus does not participate in dimerization. Surface plasmon resonance has revealed that C-terminus truncations of TnrA do not inactivate its DNA-binding activity. Contrary, increased the affinity towards DNA, confirming that C-terminus controls the DNA-binding activity in a full-length TnrA.

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In bacteria the transcriptional activity of genes is controlled by DNA-binding proteins—the transcription factors. These proteins interact with the promoter region of the gene and activate or inhibit its transcription in response to the changes in environment. In *B. subtilis* cells the transcription factor TnrA controls the activity of genes of nitrogen metabolism under conditions of nitrogen limitation [1]. As a dimer, it interacts with the palindromic sequence in the promoters of target genes and belongs to the MerR family of transcription regulators. Most of these proteins have a similar tertiary structure and control the cellular response towards the changing metal concentrations in the environment [2].

Proteins of the MerR family are present in the cells in a dimeric form and have two functional domains [2]. The N-terminal domain interacts with DNA, the C-terminal is responsible for the regulatory signal transduction. Despite of the high homology between the DNA-binding N-terminal domains of TnrA and MerR proteins, the C-terminal domain of TnrA is significantly shorter than that of other MerR proteins [3]. However, the C-terminus of TnrA interacts with GlnK and glutamine synthetase confirming its participation in the transduction of regulatory signal [3, 4].

The question about the TnrA dimerization site remains still unresolved [3, 4]. Previous studies demonstrated that the removal of up to 27 amino acids from the C-terminus of the protein does not affect protein dimerization, although it leads to the removal of an α-helix, which has a high homology to the dimerization domain of a number of MerR family proteins [2–5]. In this paper we present a model for the dimerization of the transcription factor TnrA, which is different from the dimerization of the other MerR family proteins, and also show that the C-terminal domain in the full-length TnrA controls its DNA-binding activity and is required to stabilize the TnrA–DNA complex.

EXPERIMENTAL PROCEDURES

Strains and plasmids. The cloning procedures were performed in *E. coli* XLI-Blue (*recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lac*), (Stratagene, USA). *E. coli* BL21 was used for the overexpression of recombinant proteins. pET15b vector (Novagen) replicates in *E. coli* cells, carries T7 promoter under the control of *lac*-operator and provides the overexpression of recombinant proteins with N-terminal 6-His-tag in *E. coli* BL21 cells. The plasmids pET15b-TnrA6, pET15b-TnrA20 and pET15b-TnrA35 were constructed earlier [4], the plasmid pET15b-TnrA43 was

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