SIGNAL TRANSDUCTION BY THE ARCB SENSOR KINASE

LACKING THE PAS DOMAIN

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Introduction. The ArcB/A (anoxic redox control) two-component signal transduction system of *Escherichia coli* modulates the expressions of numerous genes depending on the redox conditions of growth. Under an anoxic condition, the ArcB sensor kinase senses the redox state of the quinine pool and autophosphorylates and transphosphorylates its cognate response regulator ArcA. Phosphorylated ArcA (ArcA-P) can bind to its target promoters and either activate or repress their expressions. In the present study, we employed biochemical, molecular genetic, and functional genomic approaches to characterize the ArcB/A two-component signal transduction system of the rumen bacterium *M. succiniciproducens*.

Methods. From the genome sequence of *M. succiniciproducens* MBEL55E, the putative *arc* genes, *MsarcA* (MS1730) and *MsarcB* (MS1504) were identified. MsArcB and MsArcA were, overexpressed and purified in *E. coli* as His_6-tagged proteins. The *in vitro* autophosphorylation of MsArcB and subsequent transphosphorylation to MsArcA were tested by incubation with [γ-^{32}P]ATP in the presence or absence of quinone compounds or metabolites. To address whether the *arc* genes of *M. succiniciproducens* are able to substitute for the *E. coli* genes under physiological conditions, a functional complementation assay of the toluidine blue O sensitivity of *E. coli arc* mutants was performed. The putative target genes of the Arc system of *M. succiniciproducens* were determined by analyzing the transcriptome of the ArcA overexpression strain and by the *in silico* scanning of the entire genome sequence with the position weight matrix of the ArcA binding sequence developed for *E. coli.*

Results. The ArcB and ArcA of *M. succiniciproducens* show 48% and 73% of amino acid sequence identity with the *E. coli* ArcB and ArcA proteins, respectively. MsArcB lacks the PAS domain, which contains the two redox-active cysteine residues critical for redox signaling by the *E. coli* ArcB protein (Fig. 1). Moreover, it has been shown that unlike the *E. coli* ArcB, the *in vitro* kinase activity of ArcB of *M. succiniciproducens* is not affected by quinone compounds or by anaerobic metabolites. According to transcriptome profiles of ArcA overexpressed strain, the majority of 79 repressed genes were involved in energy metabolism and carbohydrate transport and metabolism, while the majority of 82 induced genes were involved in hypothetical or unknown functions.

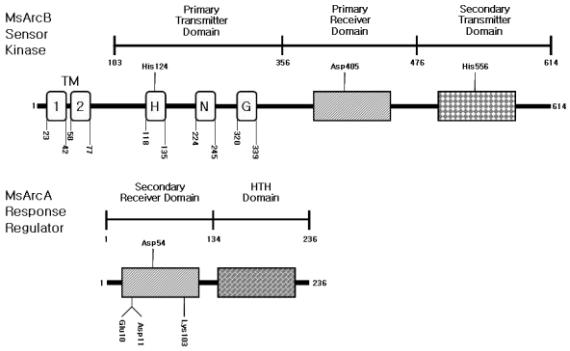

Fig. 1. Schematic representation of MsArcB and MsArcA proteins.

Conclusions. Our results taken together indicate that the Arc system of *M. succiniciproducens* may be involved in adaptive redox dependent gene regulation in response to signals other than quinone compounds, as suggested based on an *in vitro* study of recombinant Arc proteins. This system may be responsible for a stress response which requires a central metabolism reduction and the induction of mechanisms of recovery from damage. Further molecular genetic and functional genomic characterizations of the predicted ArcA target genes in wild-type and *arc* mutant strains under various growth conditions are necessary to understand the physiological role of the Arc system in *M. succiniciproducens*.

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