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The RamA-RamB System: A Carbon Source-Dependent Regulatory Network in *Corynebacterium glutamicum*

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Bacteria in general possess carbon source-dependent regulatory networks controlling specific reactions and/or whole pathways within the cell. These networks allow sequential or parallel metabolization of available substrates and adapt the central metabolism to anabolic and catabolic requirements under a given condition. Carbon source-dependent global regulatory networks have been studied and are well known in some model organisms, such as *Escherichia coli* and *Bacillus subtilis.* However, although industrially of significant interest, much less is known about such networks in *Corynebacterium glutamicum.*

C. glutamicum is a non-pathogenic, aerobic Gram-positive soil bacterium that is widely used for the large-scale production of amino acids, such as L-glutamate and L-lysine. In addition, the organism has gained increasing interest as a suitable model organism for the *Corynebacterineae,* a suborder of the actinomycetes which also includes the medically important genus *Mycobacterium. C. glutamicum* is able to grow on a variety of carbohydrates and organic acids as single or combined sources of carbon and energy. However, physiological studies, intracellular carbon flux quantification during growth on different substrates, classical molecular studies as well as the annotation of the whole genome sequence of *C. glutamicum* indicate that the carbon carbon source-dependent regulatory networks in this organism are very different from that what is known from other well-studied microorganisms.

One aim of our work is the identification and characterization of the cellular response of *C. glutamicum* to the availability of acetate and glucose/acetate mixtures. Based on biochemical, genetic and regulatory studies, on quantitative flux determinations and on genome-wide comparative expression analyses, there is considerable knowledge on the enzymes and genes involved in acetate metabolism of *C. glutamicum* (reviewed in ref. 1 and 2). The utilization of acetate involves its uptake and subsequent activation to acetyl-CoA and, when acetate is the sole carbon substrate, also requires the operation of the glyoxylate cycle as anaplerotic pathway and of gluconeogenesis. Accordingly, the key enzymes of acetate activation, acetate kinase (AK) and phosphotransacetylase (PTA), those of the glyoxylate cycle,

isocitrate lyase (ICL) and malate synthase (MS), and also PEP carboxykinase (PEPCk) as initial gluconeogenic enzyme have been shown to be essential for the growth of *C. glutamicum* on acetate as the sole carbon and energy source. All five enzymes are coordinately up-regulated in the presence of acetate in the growth medium and, as shown by classical techniques as well as by DNA microarray technology, this regulation is due to transcriptional control of the respective pta-ack operon and the *aceA, aceB* and *pck* genes.

Using cell extracts of *C. glutamicum* and employing DNA affinity chromatography, mass spectrometry, and peptide mass fingerprinting, we identified two regulatory proteins, designated as regulators of acetate metabolism A and B, i.e. RamA and RamB (3, 4). Growth experiments, enzyme determinations and transcriptional fusion experiments with the wildtype of *C. glutamicum* and with *ramA*- and *ramB*-deletion mutants indicate that RamB represents a repressor and RamA an activator of the genes encoding enzymes involved in acetate metabolism. The consensus binding motifs for both regulators were identified and further analyses revealed strong evidence for an autoregulation of both RamA and RamB. The transcript profiles of the *ramA* and *ramB* mutants grown on different substrates, the finding of consensus binding motifs in front of a variety of genes encoding enzymes of the central metabolism and subsequent electrophoretic mobility shift analyses furthermore suggest that RamA and RamB bind to and/or control a variety of genes including those for some enzymes involved in catabolic pathways. All these results indicate that RamA and B are central regulators of the carbon metabolism in *C. glutamicum,* i.e., that the RamA-RamB system represents a carbon source-dependent global regulatory network in *Corynebacterium glutamicum.*

References

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