S7-3

The Transcriptional Regulatory Repertoire of Corynebacteria: from Individual Genes to Reconstructed Regulatory Networks

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The genus *Corynebacterium* includes a diverse collection of gram-positive microorganisms of great biotechnological importance, such as *C. glutamicum* and *C. efficiens*, as well as serious human pathogens, such as *C. diphtheriae* and *C. jeikeium*. Although genome sequences of the respective species have been determined recently, the knowledge about the repertoire of transcriptional regulators and about the architecture of regulatory networks is scarce. We applied a combination of bioinformatic tools and comparative genomic approaches to identify and characterize a conserved set of DNA-binding transcriptional regulators within the corynebacterial core genome [1]. A collection of 348 DNA-binding transcriptional regulatory genes was identified in the corynebacterial genome sequences. According to deduced amino acid sequence similarities and protein structure predictions, the DNA-binding transcriptional regulators were grouped into 25 regulatory protein families. The common set of DNA-binding transcriptional regulators present in the four corynebacterial genomes consists of 28 proteins that are apparently involved in the regulation of cell division and septation, SOS and stress response, carbohydrate metabolism and macroelement and metal homeostasis.

The knowledge about both the corynebacterial genome sequences and the transcriptional regulatory repertoire now opens the way to reconstruct the topology of transcriptional regulatory networks. To facilitate this genome-wide reconstruction, we implemented the ontology-based data warehouse CoryneRegNet that is based on a multi-layered, hierarchical and modular concept of transcriptional regulation [2]. CoryneRegNet discloses detailed information on DNA-binding transcription factors and on transcriptional regulatory interactions of corynebacteria deduced from literature-derived knowledge, computer predictions and global DNA microarray hybridization experiments. Currently, the database includes data on 57 transcriptional regulators exerting regulations on 472 genes. Accordingly, CoryneRegNet allows a pertinent data management of regulatory interactions along with the genome-scale reconstruction and visualization of transcriptional regulatory networks. These models can further be combined with metabolic networks to build integrated models of cellular function including both metabolism and its transcriptional regulation.

For comprehensive generation of genome-wide transcriptional data, we developed a DNA microarray to detect global gene expression changes in *C. glutamicum* [3]. PCR products representing 93.4% of the predicted *C. glutamicum* genes were prepared and spotted in quadruplicate onto 3-aminopropyltrimethoxysilane -coated glass slides. The applicability of the *C. glutamicum* DNA microarray was demonstrated by co-hybridisation with fluorescently labelled cDNA probes. Analysis of the technical variance revealed that *C. glutamicum* genes detected with different intensities resulting in ratios greater than 1.52 or smaller than -1.52 can be regarded as differentially expressed with a confidence level of greater than 95%.

We then applied a combination of DNA microarray hybridization, bioinformatics, *in vitro* assays, and comparative genomics to decipher the regulatory network of the conserved iron-dependent transcriptional regulator DtxR of *C. glutamicum* [4]. By performing genome-wide DNA microarray hybridizations, differentially expressed genes involved in iron metabolism of *C. glutamicum* were detected in the *dtxR* mutant *C. glutamicum* IB2103. Bioinformatics analysis of the genome sequence identified a common 19-bp motif within the upstream region of 31 genes, whose differential expression in *C. glutamicum* IB2103 was verified by real-time reverse transcription PCR. Binding of purified His-tagged DtxR protein to oligonucleotides containing the 19-bp motifs was demonstrated *in vitro* by DNA band shift assays. At least 64 genes encoding a variety of physiological functions in iron transport and utilization, in central carbohydrate metabolism and in transcriptional regulation are controlled directly by the DtxR protein. The results demonstrate that DtxR acts as a dual transcriptional regulator with a major role in controlling the expression of genes involved in iron metabolism. The DtxR protein exerts its dual regulatory function as repressor of genes participating in iron uptake and iron utilization and as activator of genes responsible for iron storage and DNA protection. Moreover, the data suggest that the DtxR protein acts as global regulator by controlling the expression of other regulatory proteins that might take care of an iron-dependent regulation of a broader transcriptional network of *C. glutamicum* genes.

References

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