TRANSCRIPTIONAL REGULATION OF THE METHIONINE BIOSYNTHESIS IN ACTINOBACTERIA AND STREPTOCOCCI.

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Summary

Motivation. An evolutionary scenario has been proposed for the regulatory mechanisms of methionine biosynthesis in gram-positive bacteria based on the distribution of T- and S-boxes in various genomes. However, some bacteria, such as *Corynebacterium glutamicum* and *Streptococcus agalactiae*, use regulatory DNA-binding proteins to control methionine biosynthesis genes instead of these RNA-based mechanisms. In this study we focused on evolution of these transcription factors in *C.glutamicum, S.agalactiae* and related species.
Results. We performed detailed analysis of the orthologous regulons for transcription factors regulating methionine biosynthesis in related genomes. We predicted two new potential members of the methionine biosynthesis pathway in several genomes of corynebacteria, and identified potential binding signals for methionine transcription factors in some relatively distant and diverse genomes, such as *Bifidobacterium* and *Brevibacterium*.

Introduction

Methionine is an essential amino acids and the universal N-terminal amino acid of proteins, and because of that biosynthesis of methionine is extensively studied in various organisms to allow for their future application in biotechnological production of the methionine. Accumulating experimental data allowed us to describe a potential scenario for evolution of methionine biosynthesis regulation in bacterial genomes (Rodionov et al., 2004). As most gram-positive bacteria use T- and S-box RNA-based mechanisms of methionine biosynthesis regulation, this scenario mostly relies on the distribution of T- and S-boxes in various genomes.

Nevertheless, some gram-positive bacteria regulate the methionine biosynthesis pathway by DNA-binding regulatory proteins. These bacteria, among others, include *Corynebacterium glutamicum* and *Strepcococcus agalactiae* species, that are the focus of this study. Transcriptional regulation of the methionine biosynthesis in the *Corynebacterium glutamicum* genome is well studied (Rey et al., 2005). The regulatory protein is McbR (a member of the TetR protein family) with the binding site consensus TAGAC-N6-GTCTA. The operon structures for members of the McbR regulon also were predicted.

The transcriptional regulator of the methionine biosynthesis in *Streptococcus agalactiae* is MtaR, a member of the LysR protein family (Shelver et al., 2003). The consensus signal for regulators of the LysR family is T-N_{11}-A (Schell, 1993). The predicted binding signal for the methionine regulatory protein in streptococci is

TATAGTTtnaAACTATA (Rodionov et al., 2004). As no binding sites of MtaR have been characterized in experiment, it is not clear whether this is the signal of MtaR.

In this study we analyzed the evolution of the candidate MtaR and McbR regulons in an attempt to extend the evolutionary scenario for methionine regulatory mechanisms.

Methods and Algorithms

Complete and partial sequences of bacterial genomes were extracted from GenBank (Benson et al., 1999).

For identification of orthologous genes and site patterns, the Genome Explorer program (Mironov et al., 1999) was used. SignalX (Mironov et al., 2000) was used to construct nucleotide weight matrices. Multiple sequence alignments were done using ClustalX (Thompson et al., 1997). Phylogenetic trees of proteins were constructed by the maximum likelihood method implemented in PHYLIP (Felsenstein, 1981).

Results and Discussion

Orthologs of McbR are present in four closely related genomes of genus *Corynebacterium*: *C.glutamicum*, *C.diphtheriae*, *C.efficiens* and *C.jeikeium*, and in three more distant genomes of other Actinomycetales: *Nocardia farcinica*, *Streptomyces coelicolor* and *Leifsonia xyli*. As the operon structure for members of the McbR regulon in *C.glutamicum* was already predicted (Rey et al., 2005), we focused on orthologous regulons in other genomes. The comparison of the operon structures reveals multiple positional rearrangements even in the closely related genomes. All observed rearrangements could be classified as follows:

1. Nonorthologous replacement (threonine synthase and alkansulfonate monooxygenase)
2. Multiple operon rearrangements (mostly rearragements of the gene order in operons)
3. Duplications creating paralogs
4. Loss of orthologs (mostly in parasitic genomes)
5. Absolutely conserved operon structure was observed only for two of 22 operons, both are monocistronic.

Overall, even in closely related genomes of the genus *Corynebacterium,* we observed multiple and diverse evolutionary events despite early observations of high level of the genome stability in corynebacteria (Nakamura et al., 2003).

Our analysis suggest that methionine regulators of distant genomes of *Nocardia farcinica*, *Leifsonia xyli* and *Streptomyces coelicolor* use binding signal(s) that differ from McbR signal in corynebacteria. Therefore, we concentrate on potential binding sites for McbR only in related genomes of genus *Corynebacterium.* Our observations demonstrate conservation of regulation of the methionine biosynthesis genes in all these genomes. The combined data on the analysis of regulatory regions and positional comparison allowed us to predict two new candidate members of McbR regulon. One of them potentially encodes glutamine-amidotransferase and the only explanation of its functional role in methionine biosynthesis pathway is the regulation of synthesis on the level of aspartate formation. The functional role of the second potential member of McbR regulon is much clearer, as the corresponding protein contains the methylthioadenosine (MTA) nucleosidase domain. MTA nucleosidase catalyzes the first

reaction of methionine pool restoration from MTA, the by-product of the polyamine biosynthesis pathway.
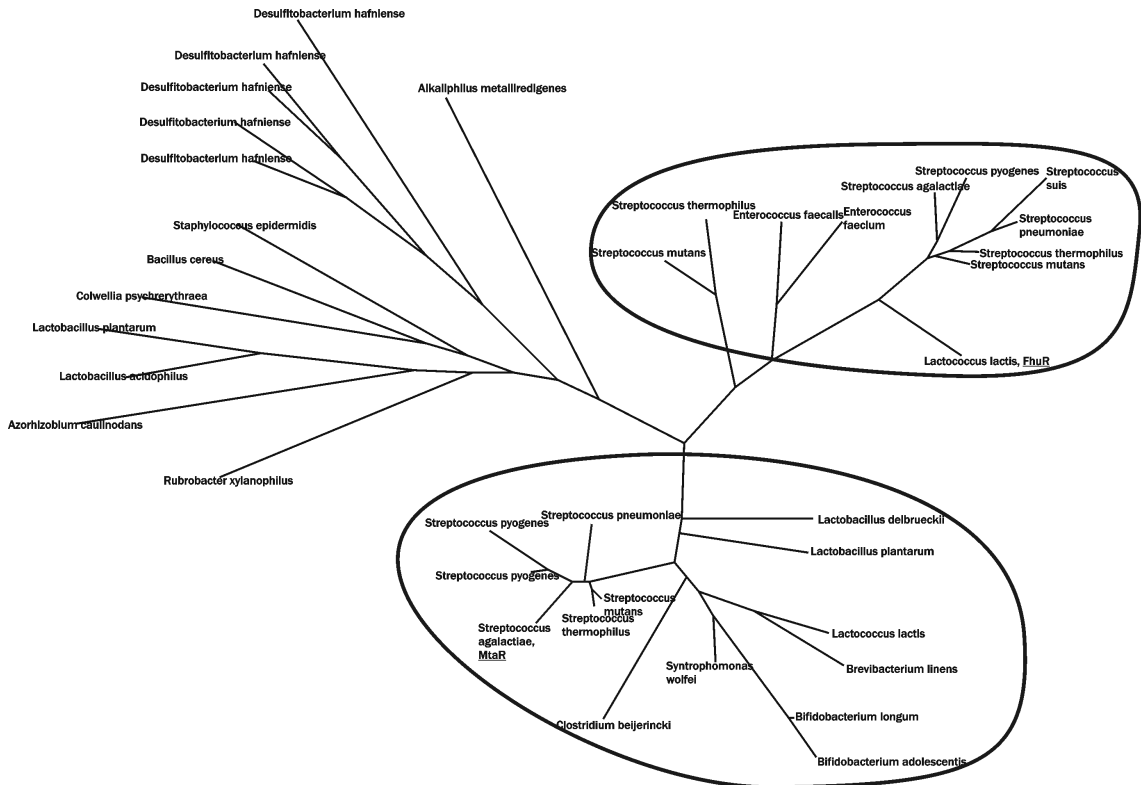

Fig.1. Phylogenetic tree of *S.agalactiae* MtaR and its relatives.

The phylogenetic tree for the MtaR protein of *S.agalactiae* (Fig.1) shows that the closest homologs of MtaR form two branches (marked in Fig.1). The branch contents differ mainly by complementary distant genomes, such as *Synthromonas*, *Bifidobacterium*, *Brevibacterium*, *Clostridium* and *Lactobacillus* for one branch, and *Enterococcus* for other. Both branches also contain the MtaR homologs from *Lactococcus lactis*, one of which has been experimentally characterized as the methionine biosynthesis regulator FhuR with the binding signal TAAAWWWTTTTA (Sperandio et al., 2005).

Overall, both branches contain regulators that potentially recognize different binding signals, ascribing the predicted streptococcal signal (Rodionov et al., 2004) to the MtaR protein of *S.agalactiae*. To verify this assumption we searched for binding signal(s) in the genomes that contain the only one of two paralogous regulators, such as *Bifidobacterium*, *Brevibacterium*, *Enterococcus* etc. Our initial observations seem to confirm the potential subdivision of specific binding signal(s) into two groups corresponding to the regulators subdivision in the phylogenetic tree. If our assumption will be proven in the following analysis, we could describe consecutive evolutionary events as methionine regulator duplication in the genome of common ancestor of streptococci followed by horizontal transfer of one of the paralogs into distant genomes.

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