EVOLUTION OF THE METHIONINE BIOSYTNTHESIS IN SPTREPTOCOCCI

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Section: computational genomics

Long abstract:

Regulation of the methionine biosynthesis is extensively studied with the aim to facilitate methionine industrial production, in particular in Gram-positive bacteria. In most Gram-positive bacterial genomes, the methionine biosynthesis is controlled by the T- and S-box mechanisms both acting on the level of premature termination of transcription [Rodionov et al., *Nucleic Acids Res*., **32**: 3340, 2004]. Nevertheless, some groups of bacteria, in particular actinobacteria and streptococci, lack the T(Met)- and S-boxes and control the methionine biosynthesis by DNA-binding regulatory proteins. The methionine regulator MtaR has been described for *Streptococcus agalacticae* [Shelver et al., *J Bacteriol*, **185**:6592, 2003], but neither its binding sites, nor the number of regulated genes are known. Independent comparative genomic analysis predicted potential binding motif `TATAGTTtnaAACTATA` (MET-box) for a streptococcal methionine biosynthesis regulatory protein [Rodionov et al., *Nucleic Acids Res*., **32**: 3340, 2004]. The phylogenetic tree of the MtaR-like proteinsfrom the LysR family shows unexpected subdivision of the MtaR homologs in two branches. Both contain all genomes of the genus *Streptococcus*, but differ in some other genomes. In particular, the branch containing MtaR orthologs also includes proteins of several actinobacterial genomes, *Clostridium beijerincki*, two *Lactobacillus* spp., *Lactococcus lactis* and others. Another branch includes homologs from two *Enterococcus* genomes and also a *L.lactis* protein. The latter has been described as the

methionine/cysteine biosynthesis transcriptional regulator FhuR with the proposed binding motif TAAAWWWTTTTA [Sperandio et al., *J Bacteriol*, **187**:3762, 2005]. Thus, both branches seem to contain methionine regulators, but with different binding motifs.

Our first aim was to check whether the MET-box is indeed the MtaR-binding motif. We have analyzed the occurrences of this motif in all involved genomes and indeed observed the correlation between the presence of MET-box-like motifs and MtaR genes. Even distant actinobacterial genomes such as *Bifidobacterium longum* and *Bifidobacterium adolescentis* have candidate binding sites upstream of methionine biosynthesis genes similar to the MET-box. Similarly, genomes from the FhuR-containg branch have binding sites corresponding to the FhuR-motif of *L.lactis*. Thus, we suggest that the MET-box is the binding motif of MtaR orthologs.

The next step was to understand the necessity of two methionine regulators in streptococcal genomes. The MtaR regulon was predicted on the basis of the candidate binding site distribution in the gene upstream regions. In mainly contains genes of direct methionine biosynthesis, such as cystathionine gamma-synthase, acetyl- or succinyl-homoserine sulfhydrolase, methionine synthase etc. The FhuR regulon of *L.lactis* includes mainly the genes of the cysteine biosynthesis, in particular the genes encoding enzymes of the methionine-to-cysteine conversion. We suppose that both regulators, MtaR and FhuR, are active and functional, and control different genes involved in the methionine/cysteine metabolism. The functional subdivision seems to be not restricted to methionine for MtaR and cysteine for FhuR, as our observations suggest that in some genomes, several genes are controlled by both regulators. Such common genes include genes of the methionine ABC transporters, some genes of the glycine pathway synthesizing methionine from methylene-tetrahydrofolate, genes of the S-adenosylmethionine recycling pathway etc. Moreover, our analysis suggests existence of a possible regulatory cascade, as a candidate binding site for FhuR has been observed in the upstream region of the MtaR gene of *S.agalacticae*.

Thus, we extend the proposed evolutionary scenario of the methionine biosynthesis regulation [Rodionov et al., *Nucleic Acids Res*., **32**: 3340, 2004]. After the loss of the S-box system, the streptococcal genomes evolved two regulators for the methionine metabolism genes, one controlling mainly the methionine synthesis genes, and the other, the cysteine synthesis genes, and both alternatively controlling the secondary methionine biosynthesis pathways. Further, we suggest a cascade regulation between these two transcription factors.

Short abstract:

Regulation of the methionine biosynthesis mainly involves the premature termination of transcription, determined by the T- and S-boxes. We suggest an evolutionary scenario for the methionine biosynthesis regulation in streptococcal genomes lacking T- and S-boxes. We show that Streptococci have two transcriptional regulators, one controlling genes of the methionine synthesis, and the other, cysteine synthesis genes. These factors also form a regulatory cascade.