BACTERIAL METAL RESISTANCE SYSTEMS REGULATED BY TRANSCRIPTION REGULATORS OF THE MERR FAMILY

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Summary

Motivation: Understanding of the metal metabolism in bacteria is inseparable from investigation of metal resistance systems. One class of such systems is regulated by the MerR family of transcription regulators.

Results: We selected metal-sensing regulators belonging to the MerR family and explored genomic loci where these regulators were located. Based on these loci and several known sites, we identified candidate regulatory sites for MerR family proteins in the regulator loci and (in several cases) elsewhere in the analyzed genomes.

Introduction

Some metals, such as iron, copper, manganese, etc. are micronutrients used in the redox process, regulation of the osmotic pressure and as enzyme components. Other metals are not essential. However, even essential metals such as zinc and copper are toxic at high concentrations. To protect themselves from dangerous environment, bacteria have different mechanisms of cell resistance to toxic metals that involve permeability barriers, intra- and extracellular sequestration, efflux pumps, enzymatic detoxification and reduction.

One class of transcription factors regulating the metal resistance in bacteria is the MerR family, named after the regulator of mercury resistance. MerR-family proteins regulate systems of mercury detoxification (MerR), resistance to zinc (ZntR), copper (CueR and HmrR), cadmium (CadR) (Brown et al., 2003). All known MerR family regulators bind to palindromic sequences located between the –35 and –10 promoter boxes. In this study we analyzed systems of resistance to high concentration of copper, cadmium and zinc regulated by the members of the MerR family.

Materials and Methods

MerR-family proteins were retrieved from the SMART database (domain accession number SM00422) (http://smart.embl-heidelberg.de/). Multiple sequence alignments were done using the CLUSTALX program (Thompson et al., 1997). Phylogenetic trees were constructed using the program PROML from the PHYLIP package (the maximum likelihood method) (Felsenstein, 1996). A simple iterative procedure implemented in the software package GenomeExplorer was used to construct a profile from a set of upstream gene fragments and to search for possible regulatory sites in genomic sequences (Mironov et al., 2000). The positional nucleotide weights in these profiles were defined as (Mironov et al., 1999):

\[ W(b,k) = \log \left[ N(b,k) + 0.5 \right] - 0.25 \sum_{i=A,C,G,T} \log \left[ N(i,k) + 0.5 \right] \]

where \( N(b,k) \) denoted the count of nucleotide \( b \) at position \( k \). The score of a \( L \)-mer candidate site was calculated as the sum of the respective positional nucleotide weights:

\[ Z(b_1...b_L) = \sum_{k=1,...,L} W(b_k,k) \]
Results and Discussion

109 out of 503 MerR family members were selected as metal-sensing based on the presence of at least two out of three conserved cysteine residues required for the cation binding (Brown et al., 2003). The selected regulators were re-aligned and a phylogenetic tree was constructed. The branches containing known regulators CadR, ZntR, CueR, HmrR, MerR were identified on the tree, while several branches contained no regulators with known specificity.

In many cases, loci, harboring metal-sensing regulators are divergons containing two genes: one of them encoding a MerR-family transcriptional regulator and the other encoding a possible transporter. Some of these transporters were experimentally verified earlier (for a review see Brown et al., 2003). In other cases, no potential regulated transporters were found in the regulator neighborhood.

Most of the transporters belong to the P-type ATPase superfamily, TC# 3.A.3 according to the TC-DB Transport Classification Database (http://tcdb.ucsd.edu/tcdb/). Almost all members of this superfamily catalyze cation uptake and/or efflux driven by the ATP hydrolysis. On the phylogenetic tree of the superfamily, all transporters co-located with MerR-family transcriptional regulators cluster on neighboring branches, and transporters of the same presumed specificity belong to the same branches. However, in several cases putative transporters exhibit no homology to the P-type ATPase superfamily, nor to any other family in the TC-DB database. All these genes are preceded by strong CadR sites. Thus, the cadmium efflux may be facilitated by two non-orthologous protein families.

After that, we investigated the identified metal resistance loci for known or candidate regulatory sites. There are seven known regulatory sites confirmed experimentally for CueR, HmrR, CadR and ZntR (two sites per regulator, except the last one, for which one site is known) (Brown et al., 2003; Brocklehurst et al., 2003). The standard mechanism of regulation by MerR is well-known: it involves protein-protein interactions between MerR and RNA polymerase, accomplished by protein-induced DNA distortions (Brown et al., 2003), and requires precise location of a palindromic regulator binding site in relation to the -35 box of the promoter with an unusually long spacer (19 or 20 bp) between the -35 and -10 promoter boxes. Thus, we searched upstream regions of genes that could be regulated by palindromic sequences located in 19 or 20 bp spacers of candidate promoters.

The typical locus structures and consensus sequences of the identified signals are listed in Table. Briefly, for CadR and its homologs the typical locus structure is a divergon including a regulator and a transporter. In the case of the CueR regulator, the typical situation is a divergon or an operon including a regulator and an efflux pump, in several cases these genes are separated. ZntR orthologs and their regulated genes usually are not linked on the chromosome. In addition to genes encoding cation transporters, other potentially regulated genes were found. In E. coli, S. typhimurium and Y. pestis, a gene encoding multicopper oxidase has a potential CueR regulatory site. Other probable CadR sites were found upstream of genes encoding putative copper chaperone (in S. typhimurium and P. putida) and cytochrome c554 (in V. vulnificus and V. parahaemolyticus). In Photorhabdus luminescens, a potential ZntR binding site was found upstream of the gene PLU4679 encoding a homolog of multidrug efflux proteins.

This analysis identified new candidate members of the metal-resistance regulons and binding signals of the MerR-family transcriptional factors. Clearly, these predictions should be verified in experiment, but they should facilitate the direct experimental approaches.

Acknowledgements

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Table. Locus structures and regulatory site consensi for metal-sensing regulators of the MerR-family

<table>
<thead>
<tr>
<th>Name</th>
<th>Genomes</th>
<th>Locus</th>
<th>Site</th>
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</thead>
<tbody>
<tr>
<td>CadR</td>
<td><em>Pseudomonas syringae</em>, <em>Pseudomonas putida</em>, <em>Pseudomonas putida</em> plasmid pWW0, <em>Acinetobacter baumannii</em> plasmid pK1H202, <em>Pseudomonas aeruginosa</em>, <em>Pasteurella multocida</em></td>
<td>Regulator (cadR)/Transporter (cadA) divergon</td>
<td>ACCCTATAGNNCNCTATGGGT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prop CadR</td>
<td><em>Rhodopseudomonas palustris</em>, <em>Pseudomonas syringae</em></td>
<td>Regulator/Transporter divergon</td>
<td><strong>ACCTGTAAGNNCTACAGGT</strong></td>
</tr>
<tr>
<td>ZnR</td>
<td><em>Escherichia coli</em>, <em>Yersinia pestis</em>, <em>Salmonella typhi</em>, <em>Salmonella typhimurium</em>, <em>Shewanella oneidensis</em>, <em>Photorhabdus luminescens</em></td>
<td>Regulator and transporter are separated except in <em>S. odensis</em>, where they are constitue an operon</td>
<td><strong>ACCTGGAGTCGACTCCAGGT</strong></td>
</tr>
<tr>
<td>ZnR</td>
<td><em>Vibrio parahaemolyticus</em>, <em>Vibrio vulnificus</em>, <em>Vibrio cholerae</em></td>
<td>Regulator and transporter are separated</td>
<td><strong>ACCTTGGAGTCGACTCCAGGT</strong></td>
</tr>
<tr>
<td>CueR</td>
<td><em>Escherichia coli</em>, <em>Salmonella typhi</em>, <em>Salmonella typhimurium</em>, <em>Yersinia pestis</em>, <em>Vibrio cholerae</em>, <em>Vibrio parahaemolyticus</em>, <em>Vibrio vulnificus</em></td>
<td>Regulator/Transporter usually constitutes an operon. In <em>P. aeruginosa</em>, <em>B. suis</em> and <em>M. loti</em> they are separated. Other co-regulated genes are separated from them</td>
<td><strong>ACCTTCCCNNNNNNGGAAGGT</strong></td>
</tr>
<tr>
<td>HmrR</td>
<td><em>Agrobacterium tumefaciens</em>, <em>Brucella suis</em>, <em>Mesorhizobium loti</em>, <em>Rhizobium leguminosarum</em>, <em>Sinorhizobium meliloti</em>, <em>Sinorhizobium meliloti</em> symbiotic plasmids pSymA and pSymB, <em>Pseudomonas aeruginosa</em>, <em>Pseudomonas syringae</em>, <em>Pseudomonas putida</em>, <em>Ralstonia solanacearum</em>, <em>Salmonella typhimurium</em></td>
<td>Regulator and transporter usually constitutes an operon. In <em>P. aeruginosa</em>, <em>B. suis</em> and <em>M. loti</em> they are separated. Other co-regulated genes are separated from them</td>
<td><strong>ACCTTCCCNNNNNNGGAAGGT</strong></td>
</tr>
</tbody>
</table>

Notes. Bold: genomes with MerR family regulators and signals identified in this study. Mercury resistance systems regulated by MerR are not shown.

References