In silico Promoter Analysis

In vitro, CovR binds to multiple AT-rich promoter sequences in the presence and absence of phosphorylation (1, 2). A consensus binding site DDHH(ATTARA)R was deduced from in vitro analysis at the CovR-regulated capsule biosynthesis locus promoter Phas, and uracil interference analyses indicated that CovR requires interaction with two adjacent thymines, whose C-5 methyl groups likely protrude into the major groove of the double helix (2). However, in vivo experiments indicated that at least four thymine pairs (in distinct binding sites) are needed for full repression by CovR at Phas (2), implying that the CovR-DNA interaction in vivo is more complex than that suggested by in vitro analyses. CovR is a member of the OmpR subfamily of response regulators, which contain a conserved winged helix-turn-helix (wHTH) DNA-binding domain (2). OmpR transcription factors exhibit versatile DNA contacts, binding to both the major and minor grooves of DNA owing to added DNA-wing interactions (3). Therefore, to identify additional conserved patterns representing putative CovR-binding domains we analyzed in silico the upstream nucleotide sequences (500 nt) of the 14 most differentially expressed genes. Each upstream promoter region was found to be AT-rich, and contain numerous dyad repeats, inverted repeats and runs of As and Ts. A common 12-nt sequence motif TTTTATDADAAHA was present in half of these CovR-regulated promoters (with one allowable mismatch). Consistent with observed expression differences, several promoters strongly influenced by covR inactivation (namely grab, hasA, mac, sagA, sda and ska), contain multiple copies of this 12-nt motif. This common motif overlaps a 16-nt motif (T^/A^TTTTTAA^/TAAAA^/C/) proposed but insufficient for CovR binding in five characterized CovR-regulated promoters (1) and the 11-nt motif (DDHHATTAA^/GAA^/G) recently proposed based on footprinting analysis of the capsule biosynthesis operon promoter Phas (2). However, the location and sequence of the 12-nt motif we identified is not well conserved in the 14 differentially regulated promoters, and no correlation exists between the degree of sequence similarity and the level of differential gene expression. In addition, this 12-nt motif could be detected with 209 occurrences (with one allowable mismatch) in SF370 promoters of genes not observed to be differentially regulated in the covR mutant in our analysis. Hence, in silico analysis of the promoter regions of 14 of the most differentially expressed genes in our analysis did not identify a conserved nucleotide motif. Phosphorylation of the CovR regulatory domain likely contributes to the determination of DNA sites with which it interacts (3, 4), and steps subsequent to phosphorylation are
also likely to be important for signaling. We expect that future experiments will help identify additional requirements for CovR binding to CovR-regulated promoters.

The functional diversity of differentially expressed genes in our analysis suggests that CovR interacts with additional regulatory networks to alter gene expression. In some cases, the differential expression observed in our analysis might result from indirect effects of \textit{covR} deletion, because inactivation of regulatory genes can have pleiotropic effects.

\textbf{References \textit{(In Silico Promoter Analysis)}}