The expression of the pur operon, which encodes enzymes of the purine biosynthetic pathway in Bacillus subtilis, is subject to control by the purR gene product (PurR) and phosphoribosylpyrophosphate. This control is also exerted on the purA and purR genes. A consensus sequence for the binding of PurR, named the PurBox, has been suggested (M. Kilstrup, S.G. Jessing, S.B. Wichmand-Jorgensen, M. Madsen, and D. Nilsson, J. Bacteriol. 180:3900-3906, 1998). To determine whether the expression of other genes might be regulated by PurR, we performed a search for PurBox sequences in the B. subtilis genome sequence and found several candidate PurBoxes. By the use of transcriptional lacZ fusions, five selected genes or operons (glyA, yumD, yebB, xpt-pbuX, and yqhZ-folD), all having a putative PurBox in their upstream regulatory regions, were found to be regulated by PurR. Using a machine-learning algorithm developed for sequence pattern finding, we found that all of the genes identified as being PurR regulated have two PurBoxes in their upstream control regions. The two boxes are divergently oriented, forming a palindromic sequence with the inverted repeats separated by 16 or 17 nucleotides. A computerized search revealed one additional PurR-regulated gene, ytiP. The significance of the tandem PurBox motifs was demonstrated in vivo by deletion analysis and site-directed mutagenesis of the two PurBox sequences located upstream of glyA. All six genes or operons encode enzymes or transporters playing a role in purine nucleotide metabolism. Functional analysis showed that yebB encodes the previously characterized hypoxanthine-guanine permease PbuG and that ytiP encodes another guanine-hypoxanthine permease and is now named pbuO. yumD encodes a GMP reductase and is now named guaC.

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