afsS is a target of AfsR, a transcriptional factor with ATPase activity that globally controls secondary metabolism in Streptmyces coelicolor A3(2)

Abstract

AfsR is a pleiotropic, global regulator that controls the production of actinorhodin, undecylprodigiosin and calcium-dependent antibiotic in *Streptomyces coelicolor* A3(2). AfsR, with 993 amino acids, is phosphorylated on serine and threonine residues by a protein serine/threonine kinase AfsK and contains an OmpR-like DNA-binding fold at its N-terminal portion and A- and B-type nucleotide-binding motifs in the middle of the protein. The DNA-binding domain, in-dependently of the nucleotide-binding domain, contributed the binding of AfsR to the upstream region of *afsS* that locates immediately 3′ to *afsR* and encodes a 63-amino-acid protein. No transcription of *afsS* in the **Δ***afsR* background and restoration of *afsS* transcription by *afsR* on a plasmid in the same genetic background indicated that *afsR* served as a transcriptional activator for *afsS*. Interestingly, the AfsR binding site overlapped the promoter of *afsS*, as determined by DNase I protection assay and high-resolution S1 nuclease mapping. The nucleotide-binding domain contributed distinct ATPase and GTPase activity. The phosphorylation of AfsR by AfsK greatly enhanced the DNA-binding activity and modulated the ATPase activity. The DNA-binding ability of AfsR was independent of the ATPase activity. However, the ATPase activity was essential for transcriptional activation of*afsS*, probably because the energy available from ATP hydrolysis is required for the isomerization of the closed complex between AfsR and RNA polymerase to a transcriptionally competent open complex. Thus, AfsR turns out to be a unique transcriptional factor, in that it is modular, in which DNA-binding and ATPase activities are physically separable, and the two functions are modulated by phosphorylation on serine and threonine residues.