

Phosphorylation of AfsR by multiple serine/threonine kinases in *Streptomyces coelicolor* A3(2)

Abstract

AfsK, a protein serine/threonine kinase, autophosphorylates on serine and threonine residues and phosphorylates serine and threonine residues of AfsR, a transcriptional activator for *afsS* involved in secondary metabolism in *Streptomyces coelicolor* A3(2). *pkaG* encoding a 592-amino-acid protein and SCD10.09 (named *afsL*) encoding a 580-amino-acid protein, both of which encode an AfsK-like protein, were transcribed throughout growth. PkaG with a histidine-tag and the kinase catalytic domain of PkaG, produced in *Escherichia coli*, autophosphorylated dominantly on threonine and slightly on serine residues. In addition, these proteins phosphorylated AfsR on threonine and serine residues. The catalytic domain of AfsL also autophosphorylated and phosphorylated AfsR, on threonine and serine residues in both cases. AfsR was thus found to be phosphorylated by multiple kinases. Disruption of the chromosomal *pkaG* gene resulted in slightly reduced production of the pigmented antibiotic actinorhodin. These findings, together with the presence of about 40 AfsK homologues and at least five AfsR homologues in *S. coelicolor* A3(2), suggest that the regulatory networks via eukaryotic-type protein phosphorylation are more diverse and versatile than we have expected.