The functions of CpkO and CpkN regulators in coelimycin synthesis and other antibiotic production pathways in *Streptomyces coelicolor* A3(2)

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ABSTRACT

Bacteria of the genus *Streptomyces* produce a multitude of secondary metabolites, many of which are pharmaceutically-relevant antibiotics, immunosuppressants and anti-cancer drugs. Secondary metabolite synthesis genes are grouped together on the chromosome into biosynthetic gene clusters (BGCs) that also encode cluster-specific regulatory proteins. Among these regulators are *Streptomyces* antibiotic regulatory proteins (SARPs), which are direct transcription activators of biosynthetic genes.

Coelimycin (CPK) is a transition growth phase secondary metabolite, produced in specific conditions by the model organism *Streptomyces coelicolor* A3(2). After its synthesis as a hydroxyaldehyde and additional enzymatic modifications, the colorless polyketide antibiotic abCPK is formed and subsequently undergoes reactions with specific compounds in the medium, loses its antibacterial properties and gives rise to the yellow coelimycins P1 and P2. Because of synthesis dependence on complex regulatory mechanisms, including quorum-sensing, carbon catabolite repression and pleiotropic regulators, coelimycin remained to be undiscovered for over 50 years of *Streptomyces* research despite being visible to the human eye.

The final putative effectors of *cpk* cluster regulation cascade are CpkO and CpkN – the two cluster-situated SARPs, which are predicted to activate the expression of Cpk type I polyketide synthase genes. Previous studies have found that CpkO is required for CPK synthesis and have linked deletion of its gene to decreased/silenced transcription of chosen *cpk* genes. However, no studies were published on CpkN – a protein belonging to the same family. Previous studies of other SARP proteins (i.e. ActII-orf4, RedD, RedZ, CdaR) have shown that these formerly „cluster-specific" regulators could also exert pleiotropic acitivities and influence other secondary metabolite synthesis pathways.

The aim of this work was to further characterize the functions of CpkO and CpkN in the regulation of coelimycin synthesis and to identify other antibiotic production pathways that are controlled, indirectly or directly, by these regulators in *Streptomyces coelicolor* A3(2). To achieve these goals, *cpkO* deletion- and *cpkN* disruption (insertion) mutants were generated and assayed for antibiotic production. Next, their proteomes were analysed using label-free, shotgun proteomics and compared to that of the wild-type strain M145. Finally, an *in vivo* reporter assay was performed to obtain detailed expression profiles of chosen *cpk* cluster genes in the wild-type and the mutant strains.

The results presented in this work confirm that CpkO is the main activator of *cpk* cluster, inducing the transcription of most of the *cpk* genes (including that of *cpkN*). CpkN, on the other hand, is responsible for activating the transcription of *scoT,* encoding a type II thioesterase necessary for CPK production. These findings, together with literature analysis, resulted in the proposal of a more-detailed mechanism of coelimycin synthesis regulation. Phenotypic and proteomic analysis revealed that CpkO and CpkN influence other antibiotic biosynthetic pathways in *Streptomyces coelicolor* A3(2), including that of actinorhodin, undecylprodigiosin and calcium-dependent antibiotic synthesis. Possible molecular background for these effects is presented and discussed.