Streptomyces coelicolor: DNA methylation and differentiation

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DNA cytosine methylation is an epigenetic modification regulating many biological processes in eukaryotes, including chromatin organization, genome maintenance and gene expression. The role of DNA cytosine methylation in prokaryotes has not been deeply investigated. In *Escherichia coli* it was recently demonstrated that cytosine methylation regulates gene expression during stationary phase [1] and that an induced state of cytosine hypermethylation leads to chromosomal DNA cleavage and cell death [2]. *Streptomyces coelicolor* is a mycelial soil microorganism, which exhibits a complex life cycle that includes three different cell types: unigenomic spores, a compartmentalized mycelium (MI) and a multinucleated mycelium (substrate and aerial mycelium, MII) [3]. The importance of DNA methylation was already described in Streptomycetes [4], but its biological role remains unknown.

The main objectives of this study are to analyze cytosine methylation pattern of *Streptomyces coelicolor* M145 during growth in liquid and on solid media, and to investigate the relationship between DNA cytosine methylation and morphological/physiological differentiation.

Cytosine methylation of total genomic DNA extracted from different developmental stages was investigated by dot-blot experiments using antibody anti-5-methylcytosine. Cytosine methylome was analyzed by BiSulphite sequencing. The biological effect of cytosine methylation was studied adding 5-aza-2'-deoxycytidine (5-AC), a hypomethylating agent, to the cultures.

Dot blot analysis revealed that the level of cytosine methylation changes during development (MI, MII and spores). Specifically, DNA methylation is higher at the MI stage than in the MII or spores. BiSulphite sequencing revealed that 30% of *S. coelicolor* genes contained a methylated motif in their upstream regions. Genes harbouring these motifs included genes related to differentiation (aerial mycelium formation and sporulation), genes involved in DNA repair/replication/condensation, as well as genes encoding proteins with unknown functions. Phenotypic analyses of cultures treated with 5-AC demonstrated that DNA methylation influences germination, aerial mycelium formation and sporulation on solid medium and antibiotic production both, on solid and in liquid medium.

Overall, our preliminary results suggest a role for DNA cytosine methylation in morphological and physiological differentiation of *S. coelicolor*. Further experiments are ongoing to demonstrate the molecular mechanisms and pathways behind the observed phenotypes.

References

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