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Characterization of a Developmental Mutant of *Streptomyces coelicolor* Using Functional Genomics

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Introduction

Streptomycetes are Gram-positive mycelial bacteria that are found ubiquitously in soil. They are a prolific source of bioactive secondary metabolites that find application as anti-infectives, anti-cancer agents and other pharmaceutically useful compounds. The biosynthesis of secondary metabolites is governed by complex regulatory cascades that involve many different families of regulatory proteins, together with extracellular and intracellular signalling molecules. Genes for the production of individual secondary metabolites are arranged in clusters, many of which also contain pathway-specific activator genes whose expression depends on pleiotropic regulators that influence the production of several secondary metabolites made by the same strain. The genome sequence of *Streptomyces coelicolor* has been determined, and contains 20 clusters predicted to specify for secondary metabolites. Under laboratory conditions secondary metabolism usually occurs in a growth phase or developmentally controlled manner, generally coinciding with the erection of aerial hyphae in surface grown cultures or being restricted to stationary phase in liquid cultures. Some genes, most notably the *bld* genes, are required for both secondary metabolism and for the formation of aerial hyphae and spores. This work focuses on one such gene, *bldA*, and uses proteomics and transcriptomics to characterize its physiological role in *S. coelicolor*.

A *bldA* mutant of *Streptomyces coelicolor* was first isolated in 1975 as part of a screen for mutants defective in aerial mycelium formation (and hence appear "bald"). Since that time, *bldA* has been the subject of many studies employing conventional molecular microbiological techniques, and the basis for its pleiotropic effects on both morphological differentiation and antibiotic production has been established. *bldA* specifies the only tRNA in the genome capable of translating efficiently the leucine codon UUA, and the effect on morphological differentiation has been linked to a TTA codon present in the transcriptional regulatory gene *adpA*. Observed defects in the production of the antibiotics actinorhodin, undecylprodigiosin and methylenomycin in the *bldA* mutant have similarly been linked

to TTA-containing regulatory genes that are present in each of the three gene clusters specifying the antibiotics. In this study we have used the post-genomic technologies of proteomics and transcriptomics to further probe the consequences of *bldA* mutation, and have revealed even greater pleiotropy in the *bldA* phenotype than previously known.

Results and Discussion

The abundance of 90 proteins in the whole-cell proteome is significantly changed as a result of *bldA* mutation. Protein extracts from samples of mycelia harvested from liquid cultures of the parent (M600) and *bldA* mutant strains were analysed by 2D gel electrophoresis, and image analysis software used to reveal significant differences between the strains. A total of 336 proteins were subsequently identified using MALDI-ToF peptide mass fingerprint analysis, 90 of which exhibited significant changes in abundance between the parent and mutant strains. Approximately 65% of these correspond to proteins that are produced in a growth phase dependent manner in the parent strain, supporting the idea that *bldA* is more significantly involved in stationary phase processes than in exponential growth. Among the differences identified, the gene product from one TTA-containing gene was completely absent in the *bldA* mutant. Protein production from the gene immediately downstream of this gene was also significantly reduced, providing evidence for polarity effects.

The abundance of proteins from many secondary metabolite clusters is altered in *ΔbldA*.

The significant changes identified between the two strains included proteins from seven secondary metabolite clusters. Proteins from the actinorhodin and undecylprodigiosin clusters were absent in the *bldA* mutant as expected, but changes were also observed in the calcium-dependent antibiotic cluster (CDA); a type III polyketide synthase cluster; two clusters specifying for different siderophore molecules; and a deoxysugar synthase/glycosyltransferase cluster producing an as yet unidentified compound. These observations reveal that *bldA* has much more wide-ranging effects on secondary metabolism than previously known.

The transcriptome and proteome data reveal clues to two new regulatory links in *S. coelicolor*. The results from analysis of the transcriptome, the whole-cell proteome and membrane proteome of the M600 and M600 Δ*bldA* strains provided information that suggested the existence of previously unidentified regulatory links involving TTA-containing genes. Follow-up experiments analysing mutant strains in which candidate genes had been knocked out confirmed the hypotheses derived from the –omics data:

•*bldA* controls the secreted trypsin-protease inhibitor SCO0762 via the TTA-containing regulatory gene *adpA*.

•*bldA* controls a cluster of genes of unknown function via the TTA-containing regulator SCO4263.

Transcriptomics reveals a major change in transition phase physiology in Δ*bldA*.

Genes encoding ribosomal proteins show a characteristic transient increase in transcription during transition phase in the parent strain M600. However, in the Δ*bldA* mutant this increase was not observed indicating a significant general alteration in the physiology of the organism. The mutant strain is unable to translate mRNA containing UUA codons (due to the absence of bldA-tRNA), and it therefore seems likely that ribosomes in the *bldA* mutant will stall during translation of such messages. We propose that this could lead to an abnormal pattern of synthesis of the stringent response factor ppGpp in the mutant that could account for the gross general changes in transcription observed during transition phase. Analysis of ppGpp levels in mycelia harvested from transition phase cultures of the parent and *bldA* mutant strains showed that ppGpp levels are significantly higher in the mutant strain, supporting our hypothesis.

Conclusions

The use of a functional genomics approach is leading to a broader understanding of the role of *bldA* in the growth and development of *S. coelicolor,* and is providing new biological insights that can be tested using more conventional molecular microbiological techniques. The results indicate that it has much more wide-ranging effects on secondary metabolism than previously known, with at least seven clusters being significantly affected in the mutant strain. It is evident that *bldA* exerts its effects on the physiology of *S. coelicolor* in several ways: directly, due to an absence of protein production from genes containing TTA codons; indirectly, by a reduction in protein synthesis from genes downstream and translationally coupled to TTA-containing genes; indirectly, via the absence of synthesis of regulatory proteins encoded by TTA-containing genes; and indirectly via induction of higher levels of synthesis of the stringent factor ppGpp.

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