Using reverse genetics & genome data to identify transcriptional regulators

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Introduction

The model system for this project is the production of the antibiotic actinorhodin by *Streptomyces coelicolor*. This is an ideal system for using reverse genetics to establish regulatory links as (i) although many mutants that are altered in actinorhodin production have been isolated, a view of the overall regulatory process is far from complete, (ii) the genome of *S. coelicolor* has been completely sequenced, and (iii) the production of actinorhodin is exquisitely sensitive to growth and environmental conditions. Furthermore, evidence suggests that often the regulation of antibiotic production converges to control expression of a pathway-specific regulatory gene. This corresponds to an activator called *actII-ORF4* in the actinorhodin system. Our approach to establish the regulatory system that controls actinorhodin production is to assume that the expression of *actII-ORF4* is controlled, at least in part, by proteins binding upstream of, or overlapping, its promoter, and to use biochemical means to isolate and characterise such DNA-binding proteins.

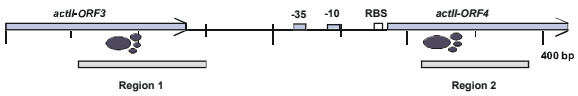

Fig. 1. Location of footprints (sites of protein binding) in relation to the promoter and RBS of *actII-ORF4*.

Binding to the promoter region of *actII-ORF4*

Using electrophoretic mobility shift assays, we have identified an ammonium sulphate fraction that can specifically shift a 420-bp fragment containing the *actII-ORF4* promoter to two new positions. Deletion analysis of the *actII-ORF4* fragment has revealed that binding is to two non-overlapping regions (named Regions 1 & 2) that flank the known position of the *actII-ORF4* promoter (Fig. 1). Moreover, competition experiments have shown that each of these regions can compete with the other, indicating that these regions contained a binding site for the same factor. The actual sites of binding within the two regions have been determined using gel *in situ* phenanthroline-Cu^{2+} footprinting. The results for one DNA strand of Region 2 are shown in Fig. 2. The location of the binding sites in relation to the promoter (Fig. 1) is suggestive of a regulatory system than involves DNA looping. Moreover, the location of a binding site well within the coding sequence of a gene (as is the case for Region 2, Fig. 1) is a strong indicator, at least in enterobacteria, that the binding protein is a repressor.

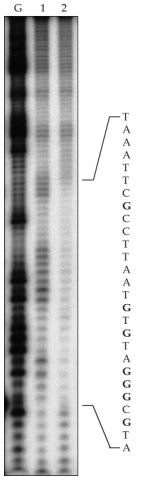

Fig. 2. Footprinting of the upper strand of Region 2

Using HPLC we have purified the DNA-binding activity by >10,000 fold. In the final elution only 4 polypeptides appeared to follow closely the peak of DNA-binding activity. The best candidate is a TetR-like repressor (SCD72A.04C), which was identified by MS analysis of a trypsin digest. The next steps are to confirm the identity of the DNA-binding activity, create a knockout mutation of the corresponding gene and then determine the effect on the transcription of *actII-ORF4* and the production of actinorhodin. We will then use reverse genetics again to identify other components in the regulation of *actII-ORF4*. This will be aided by proteome techniques we are developing to circumvent extensive purification.

Collaborators

Eriko Takano and Keith Chater, John Innes Centre, Norwich

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