Aptamers selections and bioinformatics combined extend the AtrA regulon to cell morphogenesis and nutrient uptake

Bart Boomsma, David Cobley, David H. Bunka and Kenneth J. McDowall

In a previous report, we described the identification and characterisation of AtrA, which activates the transcription of *actII*-ORF4, the cluster-situated regulator of the actinorhodin biosynthetic gene cluster in *S. coelicolor*. Subsequent to the publication of this work, we found that overproduction of AtrA promotes morphological development by a mechanism independent of *actII*-ORF4 and the production of actinorhodin. This was the first evidence that AtrA regulates the expression of multiple genes.

Bioinformatic approaches have proven to be powerful tools for the identification of additional binding sites for transcription factors in genomes when the sequences of known sites are available. To obtain multiple sequences recognised by AtrA, we used systematic evolution of ligands by exponential enrichment (SELEX) to produce DNA aptamers. The sequencing of eighteen clones revealed five unique sequences that when aligned produced a consensus resembling the known binding sites in the *actII*-ORF4 promoter.

A WebLogo representation of the consensus sequence derived from AtrA aptamers.

These sequences were then used as part of the PREDetector package to screen the *S. coelicolor* genome. The promoter of *actII*-ORF4 was one of the top hits, thus validating the approach. Another binding site was found upstream of *murC*, which encodes a ligase involved in peptidoglycan biosynthesis. This provides a plausible link to the phenotypic change resulting from AtrA overproduction (see above). Potential sites were also found upstream *murC* orthologues in two other *Streptomyces* species.

Another site was found upstream of *nagE2* and binding confirmed *in vitro* using a gel-shift assay. The product of the *nagE2* gene is a transporter of N-acetylglucosamine, a nutrient inextricably linked to morphological development and antibiotic production. Transcription of the *nagE2* gene is also controlled by DasR, a globally acting repressor that has also been reported to regulate transcription of *actII*-ORF4. The overlapping regulons of AtrA and DasR are being studied in collaboration with the group of Gilles van Wezel, Leiden Institute of Chemistry. A joint research article and invited review are in the process of being submitted.

Collaborators:
Prof. Gilles P. van Wezel (Leiden Institute of Chemistry, The Netherlands)
Prof. Fritz Titgemeyer (Münster University of Applied Sciences, Germany).

Funding
This work was funded by the European Union through the Marie Curie Actions and a BBSRC grant to K. J. M.