Investigation of the transcription complex in *Acinetobacter baylyi* ADP1 and the identification of a novel small acidic transcription factor AtfA

**Ryan Withers**
BBiotech (Hons)

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*Department of Biological Sciences*
*University of Newcastle, Australia*
Statement of Originality

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Ryan Withers
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Abstract

Transcription is an essential process by which DNA is transcribed into RNA by the multi-subunit enzyme RNA polymerase (RNAP). In prokaryotes, a single species of RNAP is responsible for the transcription of all forms of RNA (mRNA, rRNA and tRNA). Previous studies have shown that RNAP forms an extensive network of protein-protein interactions that help regulate the efficiency of transcription. Little is known about transcription and its regulation in the *Acinetobacter* spp. This group of bacteria have become notorious in recent years for their ability to rapidly acquire antibiotic resistance and hence form recalcitrant infections. This project aimed to investigate the protein-protein interactions which occur with RNAP during transcription in *Acinetobacter baylyi* ADP1 to determine if the basic components of transcription complexes are conserved in this highly diverged group of the γ proteobacteria.

The characterisation of the *A. baylyi* ADP1 transcription complexes using affinity purifications demonstrated that the protein interaction network was extensive and similar to that of well-studied model organisms such as *Escherichia coli* (Gram negative) and *Bacillus subtilis* (Gram positive). In total 233 proteins were identified, of which 13 were known, by similarity, to be transcription-related. The transcription terminator protein Rho was shown to interact directly with RNAP, confirming it forms a genuine component of the transcription complex during exponential growth. Surprisingly, and contrary to findings from previous studies, the data from this project provided evidence that Rho is essential for viability in *Acinetobacter* spp.
Eight potential transcription factors identified during affinity purifications were screened using GFP localisation studies. Of these, the hypothetical protein ACIAD2924 was shown to localise along with RNAP to the nucleoid of the cell. Affinity chromatography confirmed a direct but weak interaction between ACIAD2924 and RNAP. Subsequently ACIAD2924 was renamed to AtfA (Acidic Transcription Factor A), due to its acidic nature.

Initial characterisation of the novel transcription factor, has provided information to hypothesise the potential functions of AtfA. DNA bandshift assays illustrated that AtfA was able to displace DNA from core RNAP but not the holoenzyme, suggesting a role in the elongation phase of transcription. The AtfA deletion strain displayed a morphological phenotype whereby a significant increase in cell size was observed. RNAseq showed that 522 genes were differentially expressed in the absence of AtfA. Interestingly, genes involved in motility (type IV pili) as well as the type VI secretion system were shown to be down-regulated. The deletion strain showed a significant reduction in twitching motility and ability to form a biofilm. AtfA’s involvement in biofilm formation has great clinical significance as a potential target for the development of compounds which could prevent γ proteobacteria pathogens from forming biofilms and providing protection against conventional antibiotics.