Trancriptional Regulation of the *Mycobacterium smegmatis ami* Region

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The *ami* region of *M. smegmatis* confers the ability to use acetamide as energy source, encoding the acetamidase enzyme (AmiE), three regulators (AmiA, AmiD, and AmiC), as well as an ORF that may be involved in acetamide uptake (*amiS*). Transcription of *amiE* is inducible by acetamide, making the *ami* region one of the most widely used systems for inducible recombinant protein expression in *M. smegmatis*. In spite of its wide use and some results characterising the system, the protein-DNA and protein-protein interactions controlling *amiE* expression are not known. With a view to study mechanisms of gene regulation in mycobacteria and to improve heterologous expression systems based on *ami*, we have carried out assays to map molecular events surrounding *amiE* transcriptional activation. We have have used gel mobility shift assays to show that AmiA is a DNA binding protein that interacts with the *amiC-amiA*, and *amiD-amiS-amiE* regulatory regions. Our results also show that unlike other MarR type transcription regulators, the interaction of AmiA with DNA is not modulated by binding to the inducer of the system, in this case acetamide. AmiD regulates *amiE* expression without DNA binding, and its mode of regulation probably involves protein-protein interactions.

Palavras Chaves: ***Mycobacteria, Protein-DNA interaction, Transcriptional regulation***

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