IDENTIFICATION OF THE PROMOTER OF AMIDASE GENE FOR EXPRESSION OF USEFUL, MYCOBACTERIAL GENES

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The genetics of mycobacteria has lagged behind because of several reasons. Mycobacteria grow very slowly. their generation time ranging anywhere between 12-24 hrs. Mycobacteria are rather hydrophobic and tend to grow in clusters and there is difficulty in purifying individual cells for genetic analysis. Very few genetic markers have been found in mycobacteria because there is no known naturally occurring genetic exchange in mycobacteria. With the creation of genomic libraries of *M. tuberculosis* more than 50 genes have been characterised. Many of them are not expressed efficiently in *Escherichia coli* (E.coli) under the control of their own promoters, since very few mycobacterial promoters are recognised by the *E. coli* transcription machinery. This clearly shows that mycobacteria use a different system of gene regulation. Understanding the gene regulation of mycobacteria might throw light on the slow growth rate, about their persistence in a resting phase and also about their intracellular survival. Besides this if inducible or strong promoters are identified they can be used in over expression of genes coding for proteins useful in diagnosis and protection.

We chose to study the regulation of acetamidase gene of *M. smegmatis.* Acetamidase gene is the first inducible gene of mycobacteria identified by Halpern and Grossuies, purified to homogeneity and shown to be inducible by Draper. Eshwar *et al.,* has cloned and sequenced the acetamidase gene and 1.5 kb upstream sequence. A further 1.4 kb of upstream sequence has been determined by Tanya *et al.,*. They have indicated that the regulation is by positive and negative control and at the mRNA level. We wanted to further study the transcription start site and identify the actual promoter so that it can be used to express other mycobacterial genes.

We took a PCR cloning approach. We designed primers from the sequence of the upstream region of the amidase gene. The fragments were constructed by PCR technology. These were used as probes to identify the size of the amidase transcript - on a northern blot. The RNA was isolated using two different methods from induced and uninduced cultures of *M. smegmatis.* This RNA was found to be suitable for norther blot and primer extension analysis.

The norther blot revealed 2 products of 3.0 kb and 1.2 kb. These 2 bands were recognised by all four probes of the upstream amidase gene. These results suggests that the whole amidase transcript is 3.0 kb in size and is then processed further.

In order to identify the transcription start sites, primer extension analysis was carried out. Oligonucleotides (18 mers) complementary to the various regions upstream of the coding region were used in the primer extension reactions. The primers were designed to hybridise 200 bases apart since this provides optimal sensitivity in the primer extension reaction. Of 12 oligonucleotides used, 2 gave consistent products.

The two primer extension products were confirmed and the transcription start sites were deciphered by running sequencing ladder generated using the respective primers and the template. From these experiments it is clear that amidase gene is transcribed as a polycistronic message. A larger transcript of 3.0 kb is formed which is further processed at a single site to give amidase gene product of 1.2 kb and the other product coding for orfs 1,2,3. This promoter is currently being used to express important mycobacterial proteins.