Cloning of *Mycobacterium leprae* genes in streptomyces

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**Introduction**

The cloning and expression of *Mycobacterium leprae* genes in an easily cultivable host might provide the means to produce *M. leprae* proteins in large quantities and much more conveniently than by growing organisms in armadillos. It is now possible to clone and express genes of interest in a wide variety of systems, including bacteria, yeasts, plant cells, insect cells and mammalian cells. However, by far the most widely used and fully characterised host system is *Escherichia coli*. Considerable progress has been made in expressing mycobacterial genes in *E. coli*. Most of this progress, however, has been made by using expression vectors which utilise regulatory signals other than those of the mycobacterial genes (1, 4, 5). In instances where expression of mycobacterial genes from their own signals has been sought, the available evidence suggests that such signals function weakly, if at all, in *E. coli* (1, 3). A host organism which permitted the expression of mycobacterial genes from their own transcriptional and translational sequences might be expected to express a wider range of mycobacterial proteins, enable intact proteins to be expressed more readily, and, in the absence of a mycobacterial cloning system, enable basic studies on the regulation of mycobacterial gene expression to be carried out in a heterologous system.

In collaboration with Professor David Hopwood and Dr. Tobias Kieser of the John Innes Institute, Norwich, UK, we are investigating the use of *Streptomyces* as a cloning system for mycobacterial genes. *Streptomyces* has been chosen because:

a) *Streptomyces* are more closely related to the mycobacteria than *E. coli*, and like mycobacterial DNA, *Streptomyces* DNA has a high G + C content (though it should be noted that *M. leprae* DNA has a lower G + C content than that of the other mycobacteria) (1).

b) Cloning systems in *Streptomyces* are well characterised.

c) *Streptomyces* promoters are heterogenous and the organisms are able to recognise a range of prokaryotic promoters.

d) It has proved possible to express metabolic pathways, comprising of a number of clustered genes in *Streptomyces*. Although this has only so far been achieved with *Streptomyces* pathways, if it can be applied to mycobacterial pathways it might prove particularly useful in looking at target enzymes for drug activity.

**Evidence that mycobacterial transcriptional and translational signals are recognised by Streptomyces**

Kieser et al. (2) have used a transcriptional promoter probe vector and a translational fusion vector to investigate the function of mycobacterial promoters. The structures of the two plasmids are shown in Figure 1. The promoter probe plasmid pIJ424 includes a promoterless kanamycin resistance determinant nptII. If a piece of DNA with promoter activity is inserted in
the BglII site the kanamycin resistance gene is activated, and the level of resistance is believed to reflect the strength of promoter activity. Kieser and his colleagues were able to show that when DNA from BCG is cloned into this site there was strong promoter activity reflected in large numbers of kanamycin-resistant transformants and high levels of kanamycin resistance. Moreover, when this activity was compared with that in E. coli, using the promoter-probe plasmid pKK232-8, promoter activity in Streptomyces was found to be much stronger and more frequent than in E. coli.

The plasmid pIJ688 lacks not only a promoter for the kanamycin resistance gene, but also lacks a ribosome binding site and the codons for the first four amino acids (Figure 1) including the start codon. Insertion of DNA with a promoter and translational initiation signal results in expression of the gene and hence kanamycin resistance. By cloning BCG DNA into this vector they were able to show that BCG translational signals were recognised as efficiently as homologous Streptomyces signals. Production of fusion proteins was also demonstrated by Western blots (with anti-npt antibody) and by «activity» blots.

Evidence that *M. leprae* transcriptional signals are recognised by Streptomyces

Since *M. leprae* DNA has a significantly lower G + C content than other mycobacteria, and than *Streptomyces*, it could be argued that what holds for BCG DNA might not hold for *M. leprae* DNA. In order to investigate the activity of *M. leprae* promoters we adopted a similar
approach using DNA from *M. leprae* and from *M. tuberculosis* cloned into the promoter probe plasmid pIJ486. The results, compared in Figure 2 with those for *Strep. lividans* and BCG DNA, indicate that activity from the pathogenic mycobacterial promoters is also strong and frequent in *Streptomyces*.

**Subcloning of known mycobacterial genes into Streptomyces**

Colony screening of *Streptomyces* recombinants, either with antibody or with nucleic acid probes, has not as yet been developed to any extent. For this reason, rather than producing genomic libraries which would require extensive screening, we have decided to start by subcloning known *M. leprae* and *M. tuberculosis* genes into *Streptomyces*. This will enable us to develop colony screening techniques using known positives, which could then be used to screen genomic libraries. Using the λgt11 libraries prepared and kindly provided by Dr. Young and his colleagues, (4, 5) we have identified three mycobacterial genes (each encoding antigens recognised by monoclonal antibodies) which are apparently being expressed as entire genes from their own promoters. These are the 65kD protein of *M. tuberculosis* Y3150), the 65kD protein of *M. leprae* (Y3178) and 28kD protein of *M. leprae* (Y3164). We have excised the mycobacterial insert by digesting with EcoRI and then ligated this with the positive selection vector pIJ697. Two of the three λgt11 clones have internal EcoRI sites, and so for these two genes we carried out partial digests and size fractionation by agarose gel electrophoresis in order to simplify the identification of the desired clones.

The positive selection vector pIJ697 is shown in Figure 3. It consists of a *Streptomyces* plasmid and an *E. coli* plasmid joined by two polylinker restriction sites and two terminator sequences in the opposite orientation. Because of the presence of the two terminator sequences, the plasmid is only stable when they are separated by inserted DNA. Thus only plasmids which contain inserts are able to replicate and confer thiostrepton resistance on the host cells. The genes encoding the three proteins mentioned above have now been subcloned into streptomycyes using pIJ697, and their expression is being investigated by Western blot analysis.

*Figure 2. Promoter activity of mycobacterial DNA in Streptomyces.*
Having demonstrated the expression of subcloned genes in *Streptomyces*, we then propose to subclone *M. leprae* genes which are being expressed as «fusion epitopes» in the λgt11 system. To do this we will probe *M. leprae* DNA using the λgt11 epitope clone, isolate the relevant gene and clone into *Streptomyces*. In this way we hope to be able to express entire mycobacterial proteins which have not been fully expressed in *E. coli* from their own promoters.

**Cloning of genes coding for enzymes**

Screening of *E. coli* libraries for the expression of mycobacterial enzymes by complementation of auxotrophic mutations has proved unsuccessful, presumably because of the weak ac-
tivity of mycobacterial promoters in *E. coli*. Given that mycobacterial gene controls appear to work in *Streptomyces*, it is likely that genes will be able to complement mutations in *Streptomyces*. Cloning of genomic DNA from mycobacteria in *Streptomyces* vectors will allow transfer of libraries between different *Streptomyces* mutants, and the detection of genes for metabolic enzymes.

**References**