

Nucleotide sequence of the 32 kDa-protein gene (antigen 85 A) of *Mycobacterium bovis* BCG

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Proteins of the antigen 85 complex are abundantly secreted into the culture supernatant of a variety of mycobacteria (1, 2). These proteins are known to be responsible for the high affinity of mycobacteria to fibronectin (3). The 32 kDa protein (antigen 85A) appears to be a major stimulant of cellular and humoral immunity towards mycobacteria both in mice and man (4-6). The gene encoding the 32 kDa protein of *Mycobacterium tuberculosis* has recently been identified by us (7).

Comparison of this gene with the α -antigen from *M. bovis* BCG (Tokyo) (also referred to as antigen 85B or as MPB 59 protein (8)) revealed 73.8% homology between coding regions of the DNA sequences (7, 9). This figure should be corrected to 77.5% considering that both sequences have been revised since their publication (10, and this report). It is of interest that the homology is limited to the region coding for the mature protein and the carboxy-terminal part of the signal sequences.

After screening a λ gt11 BCG library (prepared from *M. bovis* BCG strain 1173P2) with a 5'-230 bp PstI fragment from our *M. tuberculosis* 32 kDa protein gene (7) (see Figure 2), we have isolated and sequenced a BCG genomic clone. The latter contains a 1299 base pair sequence corresponding to the 32 kDa-protein gene which is identical to that from *M. tuberculosis* except for a silent single nucleotide change at position 1023. It contains a 1014 base pair coding region. The deduced amino acid sequence corresponds to a 338-residue protein including a 43-residue-long putative signal peptide required for the secretion of the 295-amino-acid-long mature protein (Figure 1).

These data, together with recent nucleotide sequences (Content *et al.*, in preparation) suggest, as proposed by others (11), that one or several genes coding for the antigen 85 complex and that they are strongly conserved in *M. tuberculosis* and in *M. bovis* BCG.

Therefore the previously observed differences between the 32 kDa-protein gene from *M. tuberculosis* and that of the α -antigen from *M. bovis* BCG (Tokyo) may reflect mainly sequence

differences among members of the antigen 85 gene family, rather than differences among BCG strains as anticipated before (7).

Preliminary data indicate that the various genes described here are not clustered together within the mycobacterial genome of *M. bovis* BCG strain 1173P2.

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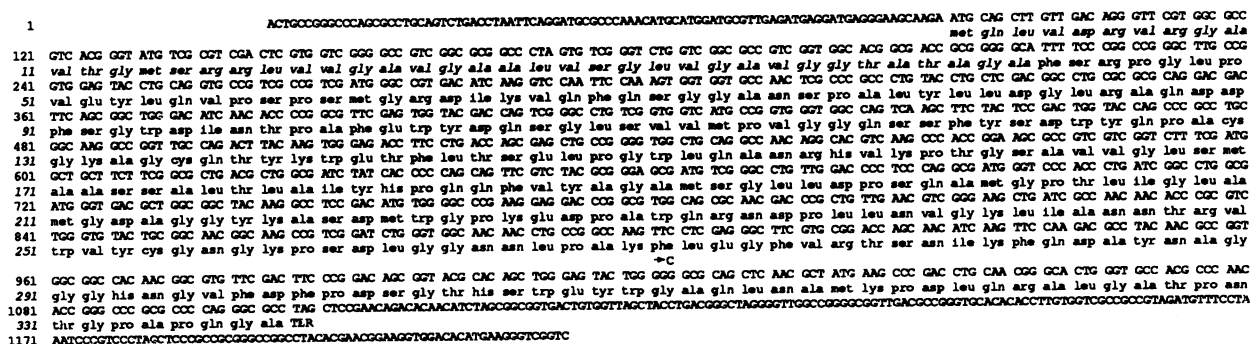


Figure 1. DNA and derived protein sequence of the 32 kDa-protein gene of *M. bovis* BCG. (strain 1173P2). The putative signal sequence is represented in italic. The sequence of the corresponding gene from *M. tuberculosis* is identical except for the indicated substitution at position 1023 (arrow).