The nucleotide sequence of the promoter, 16S rRNA and spacer region of the ribosomal RNA operon of Mycobacterium tuberculosis and comparison with Mycobacterium leprae precursor rRNA

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Mycobacterium tuberculosis H37Rv has a single rrn (ribosomal RNA) operon. The operon was cloned and a region of 1536 nucleotides was sequenced, starting 621 bp upstream from the 5'-end of the 16S rRNA coding region and continuing to the start of the 23S rRNA coding region. The 16S rRNA sequence inferred from the gene sequence was found to differ in one position from Mycobacterium bovis (nucleotide 1443) and from Mycobacterium microti (nucleotide 427). A single putative promoter was identified on the basis of similarities with the sequence of rrn operons of Bacillus subtilis and Escherichia coli. The regions of similarity include a -35 box, a -10 box, a stringent response element, antitermination signals, potential RNAase III processing sites and features of precursor rRNA secondary structure. Sequences upstream from the 5'-end of Mycobacterium leprae 16S rRNA were also investigated. Homologous schemes of secondary structure were deduced for precursor rRNA of both M. tuberculosis and M. leprae; although the principal features are common to both species there are notable differences.

Introduction

Mycobacteria are of interest because they include important pathogens such as Mycobacterium leprae and Mycobacterium tuberculosis, which have doubling times of 12 d and 1 d respectively (Shepard, 1960; Winder & Rooney, 1970). Several other mycobacteria (e.g. Mycobacterium africanum, Mycobacterium bovis and Mycobacterium microti) are very closely related to M. tuberculosis and are classified as members of the M. tuberculosis complex (Wayne, 1982). The members of this complex have a single rRNA operon and RFLP analysis has revealed that this operon and its 5'- and 3'-flanking sequences are highly conserved among members of the complex (K. E. Kempsell, I. C. E. Estrada-G, M. J. Colston & R. A. Cox, unpublished work).

This report describes further analysis of the rRNA gene family of M. tuberculosis H37Rv. The nucleotide sequence of the promoter region, the 16S rRNA coding region, the intercistronic region and the 5'-end of the 23S rRNA coding region was established. The promoter region of M. tuberculosis, which serves as a model for all members of the M. tuberculosis complex, was investigated because of our interest in the control of rRNA synthesis in slow-growing mycobacteria and its role in cell proliferation. The complete 16S rRNA sequence allows clarification of the relation of M. tuberculosis with other members of the M. tuberculosis complex and with other slow-growing mycobacteria.

In addition, we have investigated the nucleotide sequence of the putative leader sequence of the precursor rRNA (pre-rRNA) transcript of M. leprae (cf. Sela & Clark-Curtiss, 1991). The nucleotide sequence of the M. leprae rRNA operon was recently published (Liesack et al., 1990, 1991; Sela & Clark-Curtiss, 1991). Comparisons of the data for M. tuberculosis and M. leprae reveal interesting similarities and differences. Our results suggest that the single rrn operon of both species

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The nucleotide sequence data reported in this paper have been submitted to EMBL and have been assigned the accession numbers X58889, X58890 and X58891.
conforms to the general pattern established for a wide range of bacteria (for review see King et al., 1986), in which a single transcript (pre-rRNA) is processed to yield mature 16S rRNA and 23S rRNA.

**Methods**

*Materials.* All chemicals and enzymes were obtained from suppliers described previously (Cox et al., 1991). T7 sequencing kit was supplied by Pharmacia. DH5αF competent cells were supplied by Gibco/BRL. M13/pUC #1212-sequencing and #1233-reverse-sequencing primers were obtained from New England Biolabs.

*Strain and culture.* Strains of Escherichia coli K12 used in library propagation and DNA cloning were LE392 and DH5αF. Bacterial cells were grown on LB medium (Maniatis et al., 1982) at 37°C. Bacteriophage λ EMBL3 and derivatives were propagated using strain LE392 grown in LB medium plus 10 mm-MgSO4 at 37°C for all manipulations. Competent cells of strain DH5αF were used for transformations with the recombinant plasmids pUC8 and pUC18.

**Cloning of the 16S rRNA of *M. tuberculosis* H37Rv.** A Sau3A partial library of *M. tuberculosis* H37Rv genomic DNA cloned into the BamHI site of bacteriophage λ EMBL3 was provided by Dr E. Davies, Laboratory for Leprosy and Mycobacterial Research, NIMR, London. Bacteriophage plaques were transferred in duplicate onto Hybond-N nylon membranes according to the manufacturer’s instructions. Duplicate filters were then individually hybridized with either probe A [32P-labelled oligonucleotide g1 (see Table 1)] or with probe B [32P-labelled oligonucleotide cg6 (see Table 1)], which recognize respectively sequences near to the 5'- and 3'-ends of a bacterial 16S rRNA (Cox et al., 1991). Six recombinants hybridized with both probes. DNA was isolated from each of them using the 'maxi-preparative' method as described in Maniatis et al. (1982). All six clones were found to contain an insert which spanned an identical 11.6 kbp fragment of the rRNA operon characterized by the restriction map shown in Fig. 1. A representative recombinant, bacteriophage λ EMBL3-TB1, was selected for sequence analysis.

**Cloned *M. leprae* cDNA.** Clones of the *M. leprae* rRNA operon were isolated from a cosmid Lawrist 4 library (Cox et al., 1991). The putative leader sequence of precursor rRNA and part of the coding sequence was found in a 901 bp Sau3A/PstI fragment, which was sequenced by standard methods.

**Sequencing methods.** A 1-2 kbp PstI fragment containing 5'-16S rRNA gene sequences and upstream sequences was cloned into the PstI site of plasmid pUC8. A 1-1 kbp BamHI fragment containing 3'-16S rRNA sequences, the spacer region and the 5'-end of 23S rRNA was cloned into plasmid pUC18. Appropriate AluI and TaqI fragments of each of these recombinants were subcloned into pUC18 for sequence determination by the subcloning routine shown in Fig. 1. The 184 bp region located between the 3'-end of the PstI and the 5'-end of the BamHI fragment was synthesized by the PCR reaction using primers g3 and cg5 (see Table 1) and the product was isolated using GeneClean. The same product was obtained when either genomic DNA or phage λ EMBL3-TB1 DNA was used as substrate. All nucleotide sequences were determined by the dideoxy chain-termination method (Sanger et al., 1977). PCR amplified fragments were sequenced in triplicate on both strands using a modified Sequenase protocol (Winship, 1989).

Double-strand plasmid template was made single-stranded for sequencing by the NaOH-precipitation method (Murphy & Ward, 1989). Single strand template was then sequenced in triplicate using
Table 1. Deoxyribonucleotide primers

(i)-(vi), These primers, identified by roman numerals, were used in sequencing. A, B. These primers were radiolabelled at the 5'-end with $^{32}$P and used to screen the M. tuberculosis H37Rv/EMBL3 library.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Nucleotide position in M. tuberculosis 16s rRNA gene sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>tbpl (i)</td>
<td>5’(GGTGAGTCTCGGTGCCGAGATCG)3’</td>
<td>312–334</td>
</tr>
<tr>
<td>tbp2 (ii)</td>
<td>5’(GCCAGTCTAAACAAATCCCGGCT)3’</td>
<td>405–427</td>
</tr>
<tr>
<td>g3 (iii)</td>
<td>5’(GTGCCAGCAGCCGCGGTAATACG)3’</td>
<td>1126–1148</td>
</tr>
<tr>
<td>cg5t (iv)</td>
<td>5’(CGCTTGTCGCGGCCCCGCCGTAATT)3’</td>
<td>1531–1554</td>
</tr>
<tr>
<td>v9 (v)</td>
<td>5’(ACTCTGAGAAGACTGGCCGGGGGCTAATT)3’</td>
<td>1758–1781</td>
</tr>
<tr>
<td>vl 1 (vi)</td>
<td>5’(GAGTTGACGTCGTCCCCGCTTCCTTCCTCC)3’</td>
<td>1787–1813</td>
</tr>
<tr>
<td>gl (A)</td>
<td>5’(TTGGAGAGTTTGATCCTGGCTC)3’</td>
<td>627–648</td>
</tr>
<tr>
<td>cg6 (B)</td>
<td>5’(GGTACGGCTACCTTGTTACGACTT)3’</td>
<td>2105–2128</td>
</tr>
</tbody>
</table>

* See Fig. 2.

† c indicates that the nucleotide sequence is complementary to the RNA-like strand.

M13/pUC specific # 1212-sequencing and # 1233-sequencing primers and either Sequenase or T7 (Pharmacia) sequencing kits according to the manufacturer’s instructions. When necessary, sequence-specific primers (tbpl-vl 1) were used (see Table 1 and Fig. 1).

Results

The recombinant phage λ EMBL3-TBI has the same map of restriction endonuclease sites (see Fig. 1) as that established previously for the rRNA operon by analysis of genomic DNA (K. E. Kempsell, I. C. E. Estrada-G, M. J. Colston & R. A. Cox, unpublished work). The cloning strategy shown in Fig. 1 was used to establish the nucleotide sequence 621 bp upstream from the 5'-end of the 16S rRNA cistron (1536 bp), through the spacer region (276 bp) to the 5'-end of the 23S rRNA cistron (the first 105 bp), as shown in Fig. 2.

M. tuberculosis 16S rRNA coding region

The 16S rRNA sequence, inferred from the gene sequence, comprises 1536 bp. Previously Rogall et al. (1990) used PCR amplification of a 5'-portion of the M. tuberculosis H37Rv 16S rRNA gene to obtain sequence data from the coding region, namely nucleotides 117–261 (nucleotides 738–882, Fig. 2), and nucleotides 429–498 (nucleotides 1040–1119, Fig. 2). Identical sequences were obtained for M. bovis, M. bovis BCG and M. africanum (Rogall et al., 1990). The partial sequence data of Rogall et al. (1990) and the sequence presented in Fig. 2 are in agreement. Also, sequences near to the 3'-end (nucleotides 1295–1480) were obtained previously directly from 16S rRNA by the use of reverse transcriptase (Estrada-G et al., 1989). Apart from several unidentified nucleotides the sequence determined using reverse transcriptase is very similar to the data shown in Fig. 2. The primary sequence can be folded into a secondary structure which is typical of eubacteria (see Fig. 3).

Comparison of the complete 16S rRNA gene sequence of M. tuberculosis with that of M. bovis (Suzuki et al., 1988a) revealed one difference, the insertion/deletion of a C residue located in the hairpin loop region of helix 47 [nucleotides 2022–2104, Fig. 2; nucleotides 1401–1483 in the inferred 16S rRNA sequence (Fig. 3)]. The sequence for the hairpin loop is 5'CUG3' in M. tuberculosis (see also Estrada-G et al., 1989) compared with 5'CUG3' for M. bovis, as reported previously (Suzuki et al., 1988a).

The sequence of nucleotides 46–470 of the coding region of 16S rRNA gene of M. microti was obtained earlier (EMBL Data Bank accession number X58889; K. E. Kempsell, I. C. E. Estrada-G, M. J. Colston & R. A. Cox, unpublished work). Compared with M. tuberculosis, the M. microti sequence is identical except for the deletion of a T residue located at position 427 which leads to the loss of an A-T basepair in helix 17 (Fig. 3). The anti-Shine–Dalgarno sequence (Shine & Dalgarno, 1974) comprises a tract of ten pyrimidines located at the 3'-end of 16S rRNA (see Figs 2 and 3). At least nine mRNA species of members of the M. tuberculosis complex have been sequenced (for review see Dale & Patki, 1990). The putative Shine–Dalgarno sequence in each of the mRNAs is located six to twelve nucleotides upstream from the AUG or GUG start codon, and has the potential of forming five to eight basepairs with the 3'-end of 16S rRNA (see Table 2). E. coli has a shorter anti-Shine–Dalgarno sequence (seven nucleotides), and the Shine–Dalgarno sequence is closer (4–7 nucleotides) to the AUG start codon. The functional significance of these differences is not clear.
Fig. 2. Nucleotide sequence of the entire 16S rRNA gene of *M. tuberculosis* H37Rv, upstream and downstream flanking sequences, and the 5'-terminus of the 23S rRNA gene (EMBL Data Library accession number X58890). The coding sequence of the 16S rRNA gene and the 5'-terminus of the 23S rRNA gene are boxed. Nucleotides which differ from the published sequence for *M. bovis* (Suzuki et al., 1988a) are highlighted in bold with the alternative nucleotides given below. The underlined T residue (position 427) is an insertion compared with sequence for *M. microti*. The underlined C residue at nucleotide position 2064 is an insertion compared with the sequence for *M. bovis* (Estrada-G et al., 1989). * marks the putative site for the start of transcription. The 'stringent' control element is underlined. An A-T rich 8 bp repeat sequence (underlined, broken arrow) may also be a promoter element.
Fig. 3. Schematic secondary structure of *M. tuberculosis* 16S rRNA. The sequence was inferred from the gene (see Fig. 2). The scheme is based on that of Noller (1984) for *E. coli* and is annotated as described by Dams et al. (1988). The boxed nucleotides are highly conserved among eubacteria. V1, V2 etc. are variable regions. The *M. bovis* sequence (Suzuki et al., 1988a) is identical except for the deletion of a C residue (position 1443) in the hairpin loop region of helix 47.
Table 2. *Shine–Dalgarno and anti-Shine–Dalgarno sequences of the M. tuberculosis complex*

Sequences are aligned to maximize G–C basepair formation between *Shine–Dalgarno* and anti- *Shine–Dalgarno* sequences.

*N*, ranges from four to seven nucleotides in *E. coli* mRNA.

Guanine residues participating in ‘wobble’ (G–U) basepairing are denoted by use of a lowercase letter ‘*g*'.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>16S rRNA (anti-<em>Shine–Dalgarno</em>)</td>
<td>3'-UUCUCUC 5' AAGGAG - - N - - A U G</td>
</tr>
<tr>
<td>mRNA (hypothetical <em>Shine–Dalgarno</em>)</td>
<td>5' - - - - - - - - GAGGAGG - - N - - A U G</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td></td>
</tr>
<tr>
<td>16S rRNA (anti-<em>Shine–Dalgarno</em>)</td>
<td>3'-UUCUCUCUC 5'</td>
</tr>
<tr>
<td>mRNA (hypothetical <em>Shine–Dalgarno</em>)</td>
<td>5' - - - - - - - - GAGGAGG - - N - - A U G</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> mRNA (Shine–Dalgarno)</td>
<td>5' - - - - - - - - GAGGAGG - - N - - A U G</td>
</tr>
<tr>
<td>10–12 kDa antigen</td>
<td>5' - - - - - - - - A A A G G G - - N - - A U G</td>
</tr>
<tr>
<td>19–22 kDa antigen</td>
<td>5' - - - - - - - - A A A G G G - - N - - A U G</td>
</tr>
<tr>
<td>32 kDa antigen</td>
<td>5' G G G G G - - N - - A U G</td>
</tr>
<tr>
<td>38 kDa antigen</td>
<td>5' G G G G G - - N - - A U G</td>
</tr>
<tr>
<td>65 kDa antigen</td>
<td>5' - - - - - - - - G G G G A A - - N - - A U G</td>
</tr>
<tr>
<td><em>M. bovis</em> BCG mRNA (Shine–Dalgarno)</td>
<td>5' - - - - - - - - G G G G A A - - N - - A U G</td>
</tr>
<tr>
<td>α antigen</td>
<td>5' - - - - - - - - A A A G G G - - N - - A U G</td>
</tr>
<tr>
<td>18 kDa antigen</td>
<td>5' - - - - - - - - A A A G G G - - N - - A U G</td>
</tr>
<tr>
<td>23 kDa antigen</td>
<td>5' - - - - - - - - G G G G A A - - N - - A U G</td>
</tr>
</tbody>
</table>

† See Fig. 3 and Suzuki et al. (1988a).
‡ For a compilation of mRNA sequences see Dale & Patki (1990).

**Transcription of the M. tuberculosis rrn operon and processing of the transcript**

Sequence elements implicated in the control of transcription of the rrn operon and in processing the pre-rRNA transcript to form mature 16S and 23S rRNA (see Fig. 2) were identified by comparison with elements of known function first established for *Bacillus subtilis* (Loughney et al., 1983; Ogasawara et al., 1983; Stewart & Bott, 1983) and *E. coli* (Young & Steitz, 1978; Berg et al., 1989; Gourse et al., 1989; Li et al., 1984). These elements have since been recognized in a wide range of bacteria (for review see King et al., 1986).

In total, 11 putative elements were identified, namely -35 box, -10 box, the 5'-end of precursor rRNA (or start of transcription), a stem structure formed between the leader region of the transcript and the intergenic (spacer) sequences separating mature 16S rRNA and 23S rRNA, RNAase III processing sites within this stem, and also a second potential processing site in the spacer region, the antitermination signals Box A, Box B, Box C in the leader region and Box A and Box B but no Box C in the spacer region, as discussed below.

Consensus sequences established for rrn operons of *B. subtilis* include the -35 box [5'TTGAC(T/A)3'], -10 box [5'TA(T/C)(T/A)(T/A)T3'] and the site for the start of transcription (see below). The corresponding sequences for the rrn operon of *M. tuberculosis* (see Fig. 2) are -35 box (5'-TTGACT3') and -10 box (5'-TAGACT3').

The start site for transcription of the rrn operon is implicated in the growth-rate regulation and stringent control of transcription. Transcription of tRNA genes is regulated in a similar way (Duester et al., 1982). The 5'-end of transcripts from tRNA and rRNA genes have features which distinguish them from transcription of genes coding for proteins (Ogasawara et al., 1983). The established sequences include 5'GTGGTAG3' for *E. coli* tRNAeu (Duester et al., 1982); 5'GCCGCGCG3' for the *E. coli* rrnG operon (Li et al., 1984); 5'GTTGTTA3' for *B. subtilis* tRNA (Ogasawara et al., 1983); and 5'G(T/C)(T/C)G(C/T)TT3' for *B. subtilis* rrn operons (Ogasawara et al., 1983). The corresponding sequence for *M. tuberculosis* (see Fig. 2) is 5'GTTGCC3', which conforms to the general pattern.

Antitermination signals which influence the response of RNA polymerase to pause sites and terminator sites have been found in both the leader and spacer regions of the rrn operons of *E. coli* (Berg et al., 1989; Gourse et al., 1989; Li et al., 1984). The *E. coli* rrn antitermination
motif appears to be widespread (Berg et al., 1989; King et al., 1986). The elements Box A, Box B and Box C, which were first identified in bacteriophage λ, were subsequently found in rrn operons of E. coli, but in the order Box B, Box A and Box C in the leader region and Box B and Box A (but no Box C) in the spacer region (see, for example, Berg et al., 1989). The three motifs (Box B, Box A and Box C) are found within approximately 70 bp immediately following the E. coli rrnG P2 promoter (Li et al., 1984). These motifs have their homologues within 65 bp downstream from the putative start of transcription of the rrn operon of M. tuberculosis (see Fig. 2).

Box B is a region of hyphenated dyad symmetry with the potential for the RNA-like strand to form a hairpin loop. The sequence 432–446 (5'GCCCGAAGCGGGGC3'; Fig. 2) is very similar to the Box B element (5'GCCCTGAAGAAGGGC3') of the nutL gene of bacteriophage λ (Friedman & Gottesman, 1983). The Box B element tentatively identified in the spacer region (2366–2375) has a different sequence.
Young leader and spacer sequences (see Fig. 4a), described by (1983) for with 1 10-1 transcript by the method of Staden (1984), including the potential secondary structure was derived for the rrn (STGTTCTTTG3') of Fig. 2.

The Box C element has the consensus sequence STGTTGTTTG3' in the rrnG operon of M. tuberculosis (Friedman et al., 1983), STGCTCTTTA3' in B. subtilis (Ogasawara et al., 1983), and STGTGTGGG3' (the arrows indicate RNAase III cleavage sites) compared with the M. tuberculosis sequence 5'GUUGUUUGAGAA/CU/CAA3' (the broken arrows indicate possible RNAase III cleavage sites). Part of the leader region (positions 464-478) is largely repeated in the spacer region (positions 2396-2416), namely SGCGUGUUGUUGCAA-UCU (see Figs 2 and 4a). This repeat sequence is a candidate for one strand of a second stem structure (including an RNAase III processing site) formed by interaction with sequences downstream from the 3'-end of 23S rRNA (cf. B. subtilis; Ogasawara et al., 1983). In the rrn operons of B. subtilis (Stewart & Bott, 1983), Mycoplasma (Taschke & Herrman, 1986) and Streptomyces (Pernodet et al., 1989), the Box A motif is located within the stem structure close to the RNAase III processing site, as we have found for M. tuberculosis (see Fig. 4a).

The spacer region (Fig. 2) comprises 276 bp, which is the same length as the M. bovis BCG spacer (Suzuki et al., 1988a) but which is 6 bp shorter than the M. leprae spacer (Liesack et al., 1991). We did not detect tRNA genes in the spacer region (see Fig. 2). In this respect,
M. tuberculosis resembles M. bovis BCG (Suzuki et al., 1988a), M. leprae (Liesack et al., 1991), both operons of Mycoplasma capricolum (Sawada et al., 1984), the single operon of Mycoplasma hyopneumoniae (Taschke & Herrmann, 1986), Streptomyces ambofaciens (Pernodet et al., 1989), Streptomyces coelicolor (Bayliss & Bibb, 1988), Streptomyces lividans TK21 (Suzuki et al., 1988b), and eight out of ten of the B. subtilis rrr operons (Loughney et al., 1982). However, tRNA genes have been located downstream of the rrr operon of M. tuberculosis (Bhargava et al., 1990).

M. leprae precursor rRNA

The nucleotide sequence of the Sau3A/PstI fragment of the M. leprae rrr operon was established and was combined with published data for the spacer region separating the 16S rRNA and 23S rRNA genes (Liesack et al., 1991) to generate a possible secondary structure for the putative precursor rRNA (see Fig. 4b). Comparison of Figs 4(a) and 4(b) reveals that the same overall scheme of secondary structure may apply to both M. tuberculosis and M. leprae, although there are appreciable differences in detail. For example, the putative stringent response and antitermination signals, and RNase III processing sites are very similar; however, the leader sequence is longer in M. tuberculosis (208 nucleotides) than in M. leprae (191 nucleotides).

The leader sequence presented in Fig. 4(b) differs from the published sequence (Sela & Clark-Curtiss, 1991) by the presence of two additional C residues in the putative Box B antitermination signal forming a run of four consecutive C residues.

The differences in the secondary structure of precursor rRNA of M. leprae and M. tuberculosis are attributable to insertions and deletions in the M. leprae leader and spacer regions compared with M. tuberculosis (see Fig. 4c). The bihelical stem region formed between part of the leader sequence and part of the spacer region provides evidence for compensating changes; for example, deletion D1 [Fig. 4c(ii)] in the leader sequence is matched by deletion D3 in the spacer region [Fig. 4c(iii)]. The high degree of homology (see Fig. 4b) between the secondary structures proposed for M. leprae and M. tuberculosis, together with the evidence for compensating changes, lends support to the schemes proposed in Fig. 4.

Discussion

Members of the M. tuberculosis family have a single rrr operon (K. E. Kempsell, I. C. E. Estrada-G, M. J. Colson & R. A. Cox, unpublished work). They also have fewer ribosomes than many other bacteria. However, their complement of ribosomes varies according to the growth rate (Winder & Rooney, 1970). More than 80% of the RNA fraction of a bacterium is rRNA, so that the ribosome complement is reflected in the RNA:DNA ratio. In the mid-exponential phase this ratio is 2:1 for M. tuberculosis, 4:1 for Mycobacterium smegmatis and 20:1 for E. coli (Winder & Rooney, 1970), that is the complement of ribosomes are in the proportions 1:2:10.

M. tuberculosis has a single rrr operon, M. smegmatis has two (Bercovier et al., 1986) and E. coli has seven (Kiss et al., 1977). The single operon of M. tuberculosis provides about 70% of the number of ribosomes synthesized by one operon of E. coli, on the basis of the assumption that the seven operons are equivalent. The evidence that M. tuberculosis maintains growth-rate-dependent control of ribosome biosynthesis rests on the observation that the RNA:DNA ratio varies according to growth conditions whereas the ratio RNA:protein remains unchanged (Winder & Rooney, 1970). The sequence data presented above for M. tuberculosis has similarities with elements of the promoter and leader regions of the rrr operons of B. subtilis and E. coli, suggesting that they have similar mechanisms for the control of rRNA synthesis.

On the basis of its similarity with M. tuberculosis precursor rRNA, the single rrr operon of M. leprae appears functional (see also Sela & Clark-Curtiss, 1991). Indeed, the RNA:DNA ratio of M. leprae indicates that each cell contains at least 2000 ribosomes (Estrada-G et al., 1988). Thus it is unlikely that the exceptionally slow growth rate of M. leprae arises from a defect in its capacity for ribosome biosynthesis. A more likely explanation is that, for other reasons, M. leprae is incapable of independent growth and survives as an obligate parasite (for review see Wheeler, 1990).

The sequence of the rrr operon of M. bovis extending from 100 bp upstream from the start of the 16S rRNA coding region through the spacer region to the start of the 23S rRNA coding region (2007 bp) was reported previously (Suzuki et al., 1988a). There are four differences compared with the equivalent M. tuberculosis sequence. The single difference in the 16S rRNA coding region was discussed above; the three other differences are found in the spacer region [positions 2169(G/C), 2170(C/G) and 2261(A/C)] as shown in Fig. 2. These four differences in 2007 bp result in 99.8% similarity in sequence. Members of the M. tuberculosis complex are also very closely related (98% similarity in 16S rRNA sequences) to other slow-growing mycobacteria (Rogall et al., 1990; Stahl & Urbance, 1990; Cox et al., 1991). This high degree of similarity in sequence suggests that the principal features of the leader region of the M. tuberculosis rrr operon are present not only in other members of the M. tuberculosis complex but also in other slow-growing mycobacteria. We have presented supporting evidence to show that this inference is true in the case of M. leprae.
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