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Heterogeneity of *dnaB* Locus of *Mycobacterium avium–intracellulare* Complex

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Analysis of the dnaB gene, homologue of the *Escherichia coli* replicative DNA helicase DnaB, from various *Mycobacterium intracellulare* complex strains revealed their dnaB genes were heterogeneity. We found that intein was included in–frame in the dnaB locus of *M. intracellulare* and that the intein is highly similar to *M. avium* intein. Phylogenetic study showed intein sequences were remote from their own host, dnaB sequences and suggested that the horizontal transfer had occurred among *Mycobacterium avium–intracellulare* complex strains.

INTRODUCTION

The *Mycobacterium avium–intracellulare* complex (MAC) group of organisms is one of the most common causes of mycobacterial lung disease (Wallace *et al.*, 1994). Routine clinical laboratory testing of MAC organisms does not differentiate between *M. intracellulare* and *M. avium* (Wallace *et al.*, 1994). In the genus Mycobacterium, pathogens such as MAC, *M. tuberculosis*, *M. bovis* and *M. leprae* are slow growers and non–pathogens such as *M. smegmatis* and *M. fortuitum* are rapid growers. However, the genetic and biochemical basis for the differences in the growth rates between different mycobacteria and the key aspects of their respective cell cycle including DNA replication are largely unknown. When we studied on DnaB function involved in DNA replication, we found that the dnaB locus of *M. intracellulare* (MI1442) contained intein. Intein is a protein sequence that is inserted in–frame within the precursor protein and is excised during posttranslational maturation, so–called protein splicing, from the precuesor pro-

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tein. Protein splicing is associated with the ligation of exteins, the flanking regions of the inteins, and is essential to regenerate functional host protein intein (Perler, 2000). Many inteins contain endonuclease domain, which exhibit homing activity. The endonuclease is thought to be participated in gene mobility of intein coding sequences into inteinless alleles (Gimble, 2000). However, not much is available on gene mobility of intein, therefore, it is necessary to characterize intein of MAC strains. In this report, we described sequencing of intein from *M. intracellulare* and the alignment analyses of intein sequences from other Mycobacteria. Furthermore, we discussed the possibility that intein is a horizontal transfer by using phylogenetic study.

MATERIALS AND METHODS

Strains

M. avium MAC104 was obtained from Dr Luiz Bermudez at Kuzell Institute, San Francisco, USA. *M. intracellulare* mc²–76 was from Dr Raul Barletta at University of Nebraska, Lincoln, USA. *M. avium* ATCC35712, *M. avium* MA1313, *M. intracellulare* strains MI1149, MI1442, MI1522, were obtained from Dr. Richard J. Wallace, Jr., UTHCT, Tyler, TX and *M. avium* A5 was provided from Dr. Robert Husson, Children's Hospital, Boston, MA.

PCR amplification, cloning and sequencing

To amplify dnaB region, oligodeoxyribonucleotide (oligo) primers based on the 5' and 3' ends of *M. tuberculosis* dnaB sequence were used in PCR with genomic DNA from mycobacterial strains. The NdeI site was incorporated for cloning the dnaB into expression vectors. PCR was performed under the following conditions: 95 °C for 5 min; 30 cycles of 94 °C (1 min), 60 °C (1 min), and 72 °C (2.5 min), and final extension of 72 °C (10 min), then held at 4 °C. PCR products were electrophoresed on a 1% agarose gel, stained eith ethidium bromide and visualized under ultraviolet light. Amplified DNAs were cloned into pGEM–T vector (Promega) and sequenced with the ABI dye terminator cycle sequencing kit with AmpliTaq DNA polymerase in the ABI377 sequencer (Perkin–Elmer Applied Biosystem). The dnaB sequences of *M. avium* ATCC35712, *M. intracellulare* mc²–76, and MI1442 were deposited in GenBank under Accession Nos. AF259901, AF259900, and AF307984, respectively. Preliminary sequence data of *M. smegmatis* was obtained from The Institute for Genomic Research website at http://www.tigr.org.

RESULTS AND DISCUSSION

Cloning and sequencing of dnaB of *M. intracellulare*

To investigate the presence of intein sequences in the dnaB genes of MAC strains, the synthetic primers based on conserved sequence around intein insertion site in the dnaB gene were used in PCR. The sizes of PCR products with 2.5 kb (Fig. 1, lanes 2~5, 8) and 1.4 kb (Fig. 1, lanes 7, 9 and 10) were detected, indicating that the dnaB locus in one of the four strains of *M. intracellulare* strains (MI1442) harbors intein, whereas all strains of *M. avium* strains (ATCC35712, A5, MAC104 and MA1313) contained intein. Southern hybridization with dnaB–specific probes confirmed the identity of the PCR products (data

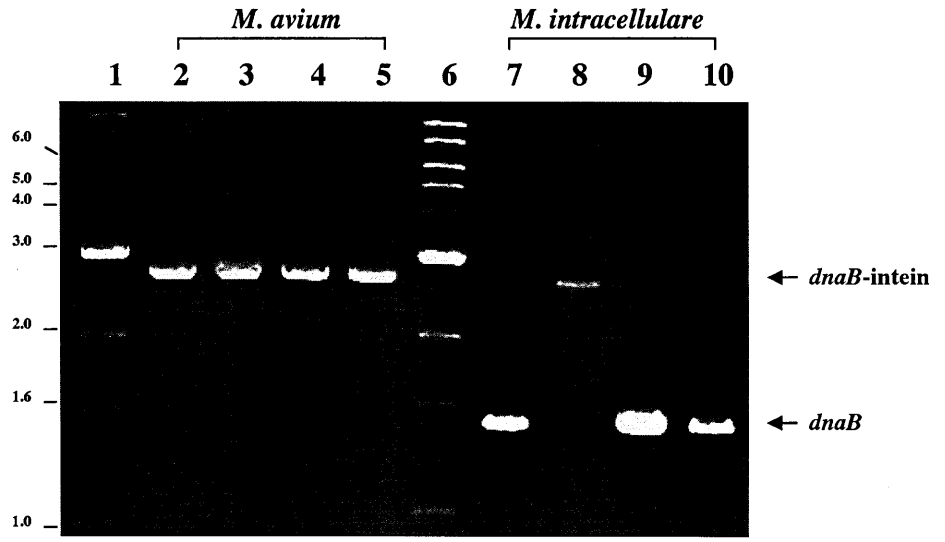

Fig. 1. Heterogeneity of the dnaB locus of MAC strains.
PCRs of genomic DNAs from different strains of MAC were performed. Lanes: 2–5: *M. avium* group; 7–10: *M. intracellulare* group; lanes 1 and 6 are 1-kb ladder. (2) *M. avium* (MA) ATCC35712; (3) MA104; (4) MA A5 and (5) MA1313; (7) *M. intracellulare* (MI) 1149; (8) MI1442; (9) MI1522 and (10) MI mc²–76. Arrows indicate that locations of dnaB with and without intein.

not shown). To verify that the PCR products prepared from *M. intracellulare* MI 1442 genomic DNA are related to intein, the dnaB region was cloned and sequenced. The nucleotide sequence analysis indicated the presence of 1002 bp DNA fragment and corresponded to an insertion of 334 amino acid long polypeptide (Fig. 2). We detected that the *M. intracellullare* sequence consists of Blocks A, N2, B, N4, F and G for splicing motif and contains several intein–specific sequence motifs designated as Blocks C, D, E and H, that are responsible for homing activities (Fig. 2) (Fsihi *et al.*, 1996; Pietrokovski, 1998). It also contains other several intein–specific sequence such as conserved C–terminal splice site –H–N–S– and putative dodecapeptide domain LAGLIDADG. A notable feature of the MAC DnaB inteins is that the intein N–terminal amino acid is Ala as opposed to Cys in *M. tuberculosis* and Ser in others (Perler, 2000). The two other families of inteins that start with Ala were discovered in the KlbA proteins and the Snf2 helicase (Perler, 2000). As shown in Fig. 2, *M. intracellulare* intein is 89% homology to *M. avium*. The DnaB inteins are related, although *M. leprae* and *M. smegmatis* inteins are missing the center region. These results indicate that identical intein is acquired in the dnaB locus of the MAC group strains.

Phylogenetic distribution of inteins

Inteins are thought to be mobile genetic elements. Homing endonuclease encoded in

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                ********** ***  ******* **  *  ****** * * ******  ****** **
M.leprae        ALALDTPLPTPTGWTAMGDVAVGDELLAVDEAPTRVVAATEVMLGRPCYEIEFSDGTVIV
M.smegmatis     ALALDTPLPTPSGWTTMGDVAVGDHLLGPDGEPTRVVADTDVMLGRPCYVVEFSDGTAIV
M.intra         ALALDTPLPTPTGWTTTGDVAVGDELLGADGKPTRVVAATEVLLGRPCYEVEFSDGTVIV
M.avium         ALALDTPLPTPTGWTTMGDVAVGDELLGDDGRPTRVVAATDVMLGRPCYEVEFSDGTVIV

                *** *** *
M.leprae        ADAQHQWPTSY-------------------------------------------------
M.smegmatis     ADAQHQWPTEH-------------------------------------------------
M.intra         ADAAHQWLTETRASRKSAQAAAAGYNRYKNQRTFAAVRTTAEIAGTLRCPTLDRRLNHSV
M.avium         ADAAHQWLTETRVSRKSAQAAAVGYNRYKNQRTFAAVRTTAEIAETLRCPAQDRRLNHSV

                                        *    *
M.leprae        -------------------------GIRTSAQLRCGLDIIAAAGSTPRH----------
M.smegmatis     -------------------------GVRITANLRAGMHTVVSAS---------------
M.intra         VNARALDLPDREFLVPPYTLGAWLGDGTSAAAQITAADPEIIMRIEAEMS-STVGTLQAR
M.avium         VNARALELPDREFLVPPYTLGAWLGDGTSAAAQITAADPEIIMRIEAEDSGAGVGTLQAR
                                 Block C

M.leprae        ------------------------------------------------------------
M.smegmatis     ------------------------------------------------------------
M.intra         LRTIGVLGNKHIPTEYLRGSETQRRELLAGLLDTDGTVTAGGAVQFSVTNQRLACDVAEL
M.avium         LRTIGVLGNKHIPTEYLRGSEAQRRALLAGLLDTDGTVTVGGAVQFSVTNQRLARDVNEL
                      Block D              Block E                Block H

M.leprae        -------------------------------------------------AGRLTTAAFMAPV
M.smegmatis     -------------------------------------------------GGRGGTALLAPA
M.intra         IVSLGYRCQTSTKRVRGRNESSSIAYTLTFSTEKKMFALERKAIHK-ERRAMAGTTRSGS
M.avium         IVSLGYRCQTSTKRVQGRSETSSIAYTLTFSTADKVFALERKAIAHKERRAVTGTSRGGS

                  *  **    * ********  * ***   ******
M.leprae        LCIDSVRRVRSVPVRCVEVDNAAHLYLAGRGMVPTHN
M.smegmatis     VQITAVRRRPSVPVRCVEVDNPEHLYLAGPGMVPTHN
M.intra         RFIVDVRPVETVAVRCVEVDNGSHLYLASRAMVPTHN
M.avium         RFIVDVRPIEPVAVRCVEVDNDSHMYLASRAMVPTHN
```

Fig. 2. Alignment of intein sequences of *M. avium*, *M. intracellulare*, *M. leprae* and *M. smegmatis* Deduced intein sequences were aligned using ClustalW multiple sequence analysis. Asterisks represent identical amino acids. Gaps in the sequences are shown as dashed lines. Conserved intein signature motif domains are underlined and are marked as C, D, E and H. Domains C and E represent putative dodecapeptide conserved domains LAGLIDADG.

intein initiates intein gene transfer to intein–less gene. In case of MAC intein, it is reasonable to propose that the dnaB–associated intein should also behave as a homing endonuclease, based on the highly conserved structural features and sequence homologies with homing endonuclease. As shown in Fig. 3, phylogenetic tree was generated from aligned sequences of three exteins and inteins in dnaB genes. Phylogenetic analysis revealed that the DnaB and inteins were separated into different groups. *M. intracellulare* intein was blanched in same group with *M. avium* and *M. leprae* inteins. Another group was generated by dnaB sequences of *M. intracellulare*, *M. avium* and *M. leprae* and was distinct from the group of intein sequences, suggesting that homing activity is believed to mediate the transposition of an intein to an intein–less allele.

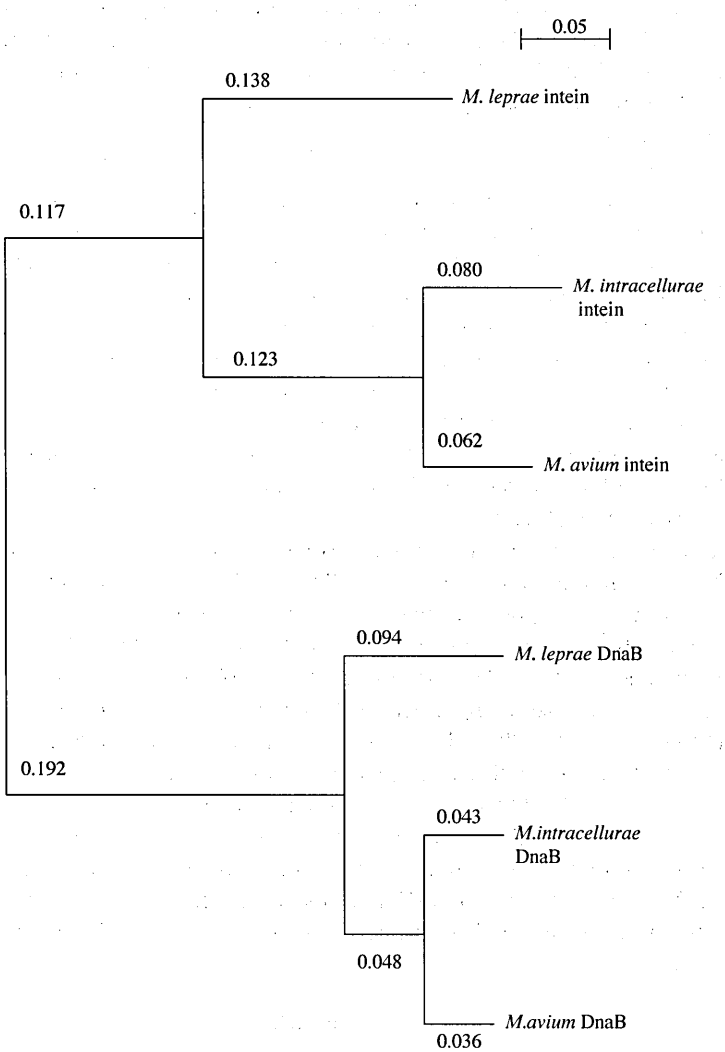

Fig. 3. Phylogenetic tree of the sequences of intein and extein in dnaB genes.
Phylogenetic tree was built upon the alignment of nucleotide sequences of intein and extein in dnaB genes which were sequenced in this study and obtained from the GenBank database, using ClustalW. The tree–building method based on Neighbour–joining was performed by the NJplot software. Numbers attached to nodes represents branch length.

The restriction sites for homing endonuclease in mycobacterium dnaB genes

With comparison of the extein insertion sites, we found that the location of this fragment is in exactly the same location (GVGK–STLGLD) in *M. avium* ATCC35712, *M. leprae* and *M. smegmatis* but differs in *M. tuberculosis* at the same location

(RESG–SLEG) as that found in *Synechocystis spp.*, the thermophile *Rhodothermus marinus* and plant chloroplast *Porphyrus purpurea* (Fsihi *et al.*, 1996; Pietrokovski, 1998). The sequences of insertion site in the *M. avium* MAC104 and A5 dnaB genes are also found to be identical to the corresponding sequence of *M. avium* ATCC35712 and *M. intracellulare* MI1442. When the nucleotide sequences of insertion sites are aligned with those of dnaB genes from *M. intracellulare*, *M. avium*, *M. leprae* and *M. smegmatis*, we found that the Lys codon corresponding to the site of insertion was AAA in *M. intracellulare* dnaB gene that reveals heterogeneity, whereas this was AAG in three of the four species harboring inteins (Fig. 4). It suggests that the corresponding homing endonuclease could recognize particular sequence and represent sequence specificity. It was also shown that the Tyr codon corresponding to the site of insertion was TAC in all gyrA genes that did not contain the intein coding sequence, whereas in three of the four species harboring inteins this was TAT in case of mycobacterial gyrA genes (Perler *et al.*, 1997). Taken together, distinction of the nucleotide sequences of the insertion sites might account for the heterogeneity, because the presence of active endonuclease motifs in *M. intracellulare* and *M. avium* dnaB inteins indicate that the MAC intein could exhibit endonuclease activity (Fig. 2).

Organisms have chance to acquire inteins. For example, plasmid and virus could carry foreign DNA to them and gene conversion, DNA repair and recombination could be occurred. In case of MAC, it is possible that recombinational events such as conjugation and transduction contribute to homing events. Conjugation is thought to be the primary route for horizontal gene transfer (Parsons *et al.*, 1998). MAC group strains are difficult to transform presumably because many of these strains contain multiple plasmids and or cryptic phages (Crawford *et al.*, 1981). It is conceivable that these plasmids harbor activities that mediate gene transfer leading to acquisition of intiens. The polyclonal nature of MAC infections (Wallace *et al.*, 1998), cell–cell contacts and Hfr conjugation in mycobacteria (Parsons *et al.*, 1998) are consistent with the above reason. The roles, if any, of intein in *M. avium* metabolism are unknown as inteins are often perceived as selfish genetic elements. The heterogeneity in MAC dnaB locus does not favor any advantageous roles for inteins to the host, although it is important to note that all *M. avium* strains

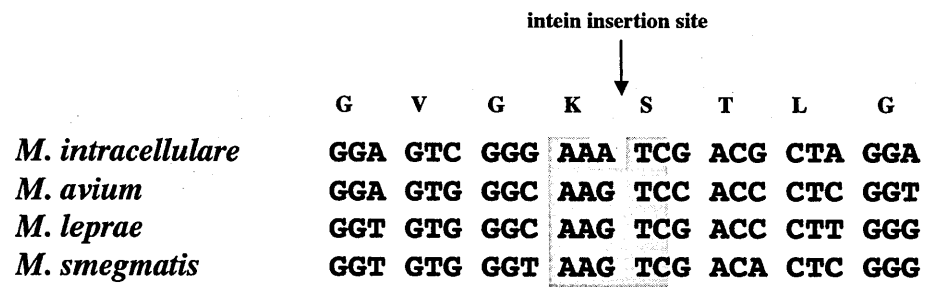

Fig. 4. Alignment of nucleotide sequences of the restriction sites in mycobacterial dnaB genes. Sequences were from alleles from dnaB genes of *M. intracellulare*, *M. avium*, *M. leprae* and *M. smegmatis*. Deduced DnaB sequence is shown above and arrow indicates intein insertion site. Conserved nucleotides in the insertion site are shaded.

examined appeared to contain intein in the dnaB gene and the majority of the MAC infections in AIDS patients are primarily due to *M. avium* (De smet *et al.*, 1996). The replicative helicase DnaB protein plays a crucial role in the DNA replication process in *Escherichia coli* (Biswas and Biswas, 1999). Assuming that the *M. avium* DnaB precursor protein undergoes splicing *in vivo* to produce functional DnaB protein, as in other intein containing precursor proteins, then it is possible to envision that the rate with which the DnaB protein is formed following splicing could influence the rate of *M. avium* DNA replication process affecting both intracellular and extracellular growth.

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