JOURNAL OF BACTERIOLOGY, Oct. 1997, p. 6378–6382 Vol. 179, No. 20
0021-9193/97/$04.00+0


# The *Mycobacterium xenopi* GyrA Protein Splicing Element: Characterization of a Minimal Intein

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Received 4 June 1997/Accepted 31 July 1997

**The 198-amino-acid in-frame insertion in the *gyrA* gene of *Mycobacterium xenopi* is the smallest known naturally occurring active protein splicing element (intein). Comparison with other mycobacterial *gyrA* inteins suggests that the *M. xenopi* intein underwent a complex series of events including (i) removal of 222 amino acids that encompass most of the central intein domain, and (ii) addition of a linker of unrelated residues. This naturally occurring genetic rearrangement is a representative characteristic of the taxon. The deletion process removes the conserved motifs involved in homing endonuclease activity. The linker insertion represents a structural requirement, as its mutation resulted in failure to splice. The *M. xenopi* GyrA intein thus provides a paradigm for a minimal protein splicing element.**

Protein splicing elements (termed inteins) were first described in 1990 as in-frame insertions in the *Saccharomyces cerevisiae VMA1* gene (9, 11). Protein splicing involves the removal of the intein from a precursor protein and the ligation of the two flanking sequences to produce a mature protein (14). Inteins are bifunctional proteins that sometimes have homing endonuclease activity, which is essential for mobility of intein genes (5, 8, 10, 13–15). Intein motifs involved in endonuclease activity (blocks C, D, and E) are found in the central region of the intein, whereas intein motifs involved in protein splicing are found in the terminal intein motifs (blocks A, B, F, and G) (14, 15). As of April 1, 1997, 41 putative intein sequences have been published or are available from public databases (14). Except for the *Porphyra purpurea* DnaB intein (150 amino acids) (16, 17), most putative inteins range in size from 335 to 548 amino acids (aa) (14).

Here, we report the characterization of a novel 198-aa *Mycobacterium xenopi* GyrA intein (*Mxe* GyrA intein) by sequence analysis, taxonomic significance, and splicing activity. This small intein has lost the residues required for endonuclease activity and may thus represent the minimal structural and functional information required for protein splicing.

## MATERIALS AND METHODS

**Identification and sequence analysis of the *M. xenopi* intein.** A collection of 29 mycobacterial species and subspecies (20) were investigated by PCR (30 cycles, with each cycle consisting of 30 s at 94°C and 30 s at 70°C) with primers H49 and H50 (7), which anneal to conserved regions in mycobacterial *gyrA* genes. The 991-bp amplification fragment from *M. xenopi* was sequenced directly from the PCR product and upon cloning into pT7T3 U13 (Pharmacia). The deducted protein sequence was aligned with other GyrA inteins (7) by using the Genetics Computer Group software and characterized by using BLAST, BLOCKS, and PredictProtein.

**Analysis of splicing and cleavage activities. (i) MIP constructs.** *Mxe gyrA* inteins were expressed in the MIP (M for maltose-binding protein, I for intein, and P for paramyosin) system (21) by substituting the *Mxe gyrA* intein for the *Pyrococcus* sp. GB-D *pol* intein-1 which was inserted in-frame between the *Escherichia coli* maltose-binding protein (MBP or M) and a fragment of *Dirofilaria immitis* paramyosin (P). PCR products were designed to include or exclude GyrA extein sequences. The partial N-extein encodes the last 65 aa of the native GyrA N-extein, and the partial C-extein encodes the first 64 aa of the native C-extein. Induction of protein expression was at 37°C for 2 h or 16°C overnight. The extent of splicing or cleavage was examined by loading soluble crude lysates on Novex sodium dodecyl sulfate-polyacrylamide gels (10 to 20% polyacrylamide) (Novel Experimental Technology, San Diego, Calif.) followed by staining with Coomassie blue or Western blot analysis with anti-MBP or antiparamyosin sera (21). When indicated, samples were incubated overnight at room temperature with 30 mM 1,4-dithiothreitol (DTT) to examine N-terminal cleavage or incubated for 5 days at room temperature to assess small amounts of slowly accumulating spliced or cleaved products. Stained gels were digitized with a Microtek Scanmaker III and analyzed with National Institutes of Health image 1.51 software for quantitation and rate determinations.

**(ii) Kan$^r$ constructs.** The splicing activity of the *Mxe* GyrA intein was also evaluated in the *E. coli*-mycobacterial shuttle vector, pYUB53 (W.R.J.) at 30 and 37°C. The intein, including the C-extein threonine codon, was amplified with Vent polymerase (New England Biolabs) to generate blunt ends and cloned into the *Ssp*I site. This procedure effectively disrupts a kanamycin resistance cassette. After electroporation (19), splicing reconstitutes kanamycin resistance in *E. coli* or *Mycobacterium smegmatis*. A control construct was made by cloning the *Mycobacterium tuberculosis recA* intein (4) in place of the *Mxe gyrA* intein.

**Nucleotide sequence accession number.** The 198-aa intein sequence has been deposited in the GenBank database under accession no. U67876.

## RESULTS

**Identification of the *Mxe* GyrA intein.** GyrA inteins were identified in 7 of 29 mycobacterial species and subspecies investigated (Fig. 1). While *Mycobacterium kansasii* type I, IV, and V, *Mycobacterium gordonae*, *Mycobacterium flavescens* type I, and *Mycobacterium leprae* presented amplification products of 1,665 bp corresponding to inteins 420 aa long, *M. xenopi* amplification resulted in a 981-bp product corresponding to a shorter intein of 198 aa. Analysis of 12 unrelated isolates of *M. xenopi* indicated that the short *Mxe* GyrA intein is characteristic of this organism.

**Sequence analysis.** Two independent clones, one containing the wild-type sequence and one carrying a C114R PCR mutation (pIMM126), were chosen for analysis. Sequence comparison to other GyrA inteins revealed an *Mxe* GyrA intein that had undergone a complex deletion event removing over 200 aa of the intein central domain between residues R107 and H143 (R107 and H365 in the *M. kansasii* GyrA intein). Moreover, a

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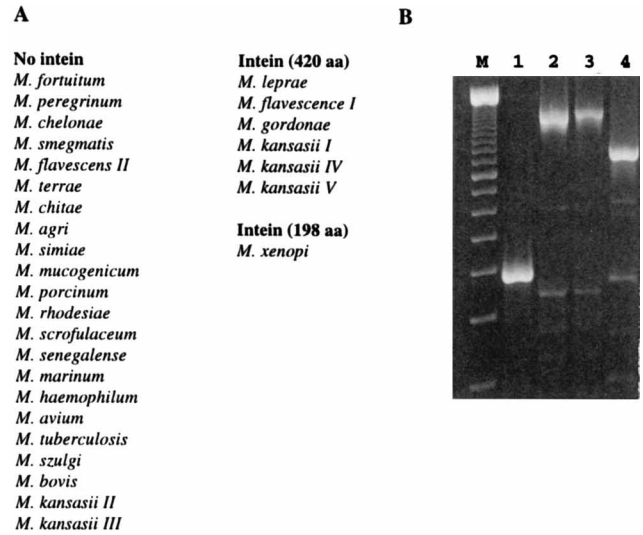


FIG. 1. GyrA inteins in mycobacteria. Investigation of 29 *Mycobacterium* species by PCR allowed the identification of intein-less alleles or the presence of long or short products resulting from in-frame insertions in *gyrA*, corresponding to intein elements of 420 and 198 aa, respectively. Lanes: M, molecular size markers; 1, *M. tuberculosis* with the 392-bp intron-less allele; 2 and 3, *M. kansasii* type I and IV, respectively, showing the 1,665-bp product; 4, *M. xenopi* with the 981-bp product.

24-aa sequence unrelated to other GyrA inteins and with no match in the database was inserted after R107 followed by a 9-aa GyrA intein sequence (V133 to E141 in *M. xenopi* or V323 to E332 in *M. kansasii*) (Fig. 2). The 9-aa preserved island motif was followed by a 35-aa GyrA intein deletion. These deletions remove four of the eight conserved intein motifs (14, 15): blocks C and E, the dodecapeptide motifs characteristic of homing endonucleases present in mobile introns and inteins that are required for endonuclease activity (5, 8, 10, 13, 14); block D, which forms part of the endonuclease catalytic site (6); and the functionally undefined block H (Fig. 2).

**Splicing activities.** The splicing activity was examined by placing the intein (with or without flanking extein sequences) into a new context, a MIP construct. The observed activities were influenced by the flanking sequences, mutation, and the expression and induction temperatures (Table 1). Small amounts of C-terminal splice junction cleavage in the absence of splicing were observed after induction of the wild-type *Mxe gyrA* intein in constructs that excluded native extein sequences (MIP). Overnight in vitro incubation of MIP at room temperature resulted in 40% C-terminal cleavage which increased to 74% at 5 days with only a limited amount of splicing (Fig. 3, lane 3). On the other hand, efficient splicing was observed after induction at 16°C, but not 37°C, in the presence of GyrA N-extein (E) sequences (MEIP) and after induction at either temperature when both native extein sequences were present (MEIEP) (Fig. 3, lanes 7 to 10). In contrast, no splicing could be demonstrated with the C114R mutant irrespective of the presence of GyrA extein sequences. Induction at either temperature of all C114R constructs except MIEP resulted in proteolytic degradation of precursors and accumulation of truncated MI and MEI (58 kDa in MIP, 63 kDa in MEIP and MEIEP) as determined by size and Western blot analysis. This proteolysis most probably resulted from the generation of a de novo protease sensitive site caused by the mutation at or near C114R. The presence of the native C-extein sequence (in the absence of the native N-extein sequence) stabilized the C114R MIEP precursor and allowed C-terminal cleavage when induced at 16°C (Fig. 3, lanes 11 and 12).

Addition of DTT reveals activation of the N-terminal splice junction in inteins beginning with Cys in the absence of the subsequent steps in the splicing pathway by cleaving the thioester bond formed at the N-terminal splice junction (22). DTT induced N-terminal cleavage of the remaining nonspliced wild-type precursors from MIP (Fig. 3, lane 4) and MIEP constructs (Table 1). However, the C114R MIEP induced at 16°C was the only mutant construct where addition of DTT resulted in N-terminal cleavage of the MIEP precursor and the previously cleaved MI protein (to form M+I) at high efficiency (Table 1).

Wild-type intein constructs (without extein sequences) in the pYUB53 system did not splice in *E. coli* or in the mycobacterial host *M. smegmatis*. In contrast, efficient splicing was observed

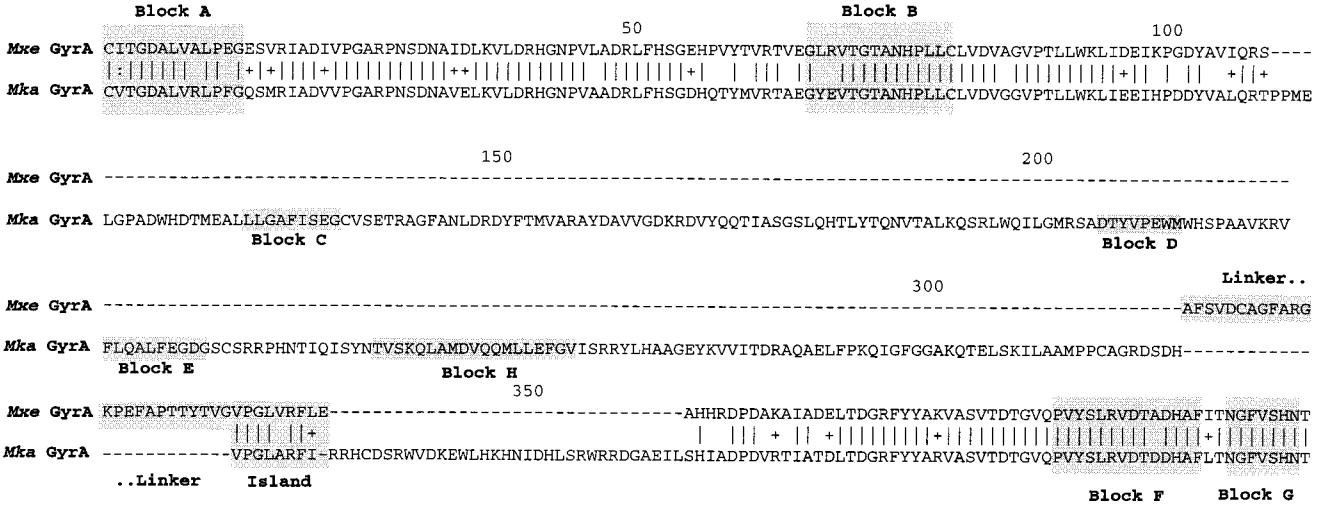


FIG. 2. Sequence alignment of *M. xenopi* (*Mxe*) and *M. kansasii* (*Mka*) inteins (including the first C-extein residue). Shared intein motifs A, B, F, and G, as well as the island motif are indicated. The *M. xenopi* intein lacks motifs C, D, E and H but has acquired a unique linker region. Deleted (-) and identical (|) amino acids and conservative substitutions and similar amino acids (:, +) are indicated.

TABLE 1. Analysis of splicing and cleavage activities of *Mxe* GyrA intein in the MBP-paramyosin chimeric system

| System and process[a] | Expected protein product(s)[b] (mass [kDa]) | % Activity or amt of product under the following assay condition[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 37°C induction | | | 16°C induction | | |
| | | Baseline | RT for 5 days | RT O/N + DTT | Baseline | RT for 5 days | RT O/N + DTT |
| WT in MIP | | | | | | | |
| Precursor | MIP (92) | 82 | 4 | 2 | 79 | 0 | 0 |
| Splicing | MP (72), I (20) | 0 | 22 | 0 | 0 | 8 | 0 |
| N-term. cleavage | M (43), IP (49) | 0 | 0 | 98 | 0 | 0 | 100 |
| C-term. cleavage | MI (63), P (29) | 18 | 74 | 0 | 21 | 92 | 0 |
| WT in MIEP | | | | | | | |
| Precursor | MIEP (98) | 64 | 3 | 22 | 51 | 0 | 3 |
| Splicing | MEP (78), I (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| N-term. cleavage | M (43), IEP (55) | 0 | 0 | 98 | 0 | 0 | 97 |
| C-term. cleavage | MI (63), EP (35) | 36 | 97 | 0 | 49 | 100 | 0 |
| WT in MEIP | | | | | | | |
| Precursor | MEIP (98) | 98 | 97 | 98 | 0 | 0 | 0 |
| Splicing | MEP (78), I (20) | 2 | 3 | 2 | 100 | 100 | 100 |
| N-term. cleavage | ME (49), IP (49) | 0 | 0 | 0 | 0 | 0 | 0 |
| C-term. cleavage | MEI (69), P (29) | 0 | 0 | 0 | 0 | 0 | 0 |
| WT in MEIEP | | | | | | | |
| Precursor | MEIEP (104) | 14 | 13 | 13 | 0 | 0 | 0 |
| Splicing | MEEP (84), I (20) | 86 | 87 | 87 | 100 | 100 | 100 |
| N-term. cleavage | ME (49), IEP (55) | 0 | 0 | 0 | 0 | 0 | 0 |
| C-term. cleavage | MEI (69), EP (35) | 0 | 0 | 0 | 0 | 0 | 0 |
| C114R mutant in MIP | | | | | | | |
| Precursor | MIP (92) | 5[d] | 3[d] | 3[d] | 50[d] | 50[d] | 50[d] |
| Splicing | MP (72), I (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| N-term. cleavage | M (43), IP (49) | 0 | 0 | 0 | 0 | 0 | 0 |
| C-term. cleavage | MI (63), P (29) | 0 | 0 | 0 | 0 | 0 | 0 |
| C114R mutant in MIEP | | | | | | | |
| Precursor | MIP (98) | 92 | 59 | 65 | 48 | 5 | 27 |
| Splicing | MEP (78), I (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| N-term. cleavage | M (43), IEP (55) | 0 | 0 | 35 | 0 | 0 | 73 |
| C-term. cleavage | MI (63), EP (35) | 8 | 41 | 0 | 52 | 95 | 0 |
| C114R mutant in MEIP | | | | | | | |
| Precursor | MEIP (98) | 3[d] | 3[d] | 2[d] | 50[d] | 50[d] | 50[d] |
| Splicing | MEP (78), I (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| N-term. cleavage | ME (49), IP (49) | 0 | 0 | 0 | 0 | 0 | 0 |
| C-term. cleavage | MEI (69), P (29) | 0 | 0 | 0 | 0 | 0 | 0 |
| C114R mutant in MEIEP | | | | | | | |
| Precursor | MEIEP (104) | 2[d] | 2[d] | 2[d] | 50[d] | 50[d] | 50[d] |
| Splicing | MEEP (84), I (20) | 0 | 0 | 0 | 0 | 0 | 0 |
| N-term. cleavage | ME (49), IEP (55) | 0 | 0 | 0 | 0 | 0 | 0 |
| C-term. cleavage | MEI (69), EP (35) | 0 | 0 | 0 | 0 | 0 | 0 |

[a] WT, wild-type *M. xenopi* intein; N-term., N-terminal; C-term., C-terminal.
[b] Identification of products was confirmed by Western blot analysis. The proteins were named as follows: M, maltose-binding protein; P, paramyosin; I, intein; E, extein. Precursors include or exclude native N- or C-extein sequences as indicated.
[c] Baseline values are the relative amounts of products observed after in vivo induction at the indicated temperature. Splicing and cleavage activities after incubation of crude lysates for 5 days or overnight are shown. Abbreviations: RT, room temperature; O/N, overnight; + DTT, with DTT.
[d] Degradation products in pIMM126 in MIP (58 kDa) and in MEIP and MEIEP (63 kDa) reacting with anti-MBP sera.

at 30°C (not at 37°C) with constructs containing only the *M. tuberculosis* RecA intein in the pYUB53 system.

## DISCUSSION

Inteins are generally considered bifunctional enzymes, capable of splicing and homing endonuclease activity. It has been proposed that these functions are independent, as mutations eliminating one activity do not eliminate the other (10). In the case of the *Mxe* GyrA intein, it is conceivable that the loss of the central region would have destabilized the intein structure and affected its protein splicing ability. However, the present report demonstrates that the 198-aa intein is capable of splicing, thus defining the large 222-aa deleted region as dispensable for this function. Positioning of the *Mxe* GyrA intein deletion is simplified because of the high degree of similarity among the GyrA inteins (7, 14). The deleted region includes all the motifs associated with endonuclease activity (blocks C, D, and E) (13–15). These data strongly suggest that inteins with both splicing and endonuclease activities fold the two func-

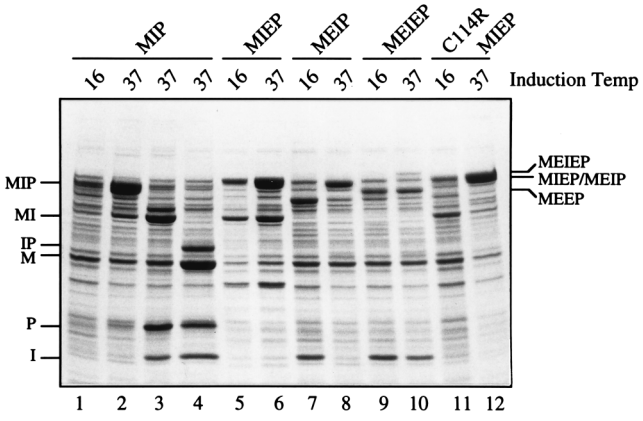


FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cleared lysates induced at the indicated temperature. In vitro incubation of lysate for 5 days at room temperature (lane 3) and in vitro incubation overnight at room temperature in the presence of DTT (lane 4) are shown. The presence of spliced products MP, MEP, and MEEP was confirmed by Western blot analysis.

tional domains independently. This hypothesis has been confirmed with the recent publication of the structure of PI-SceI, the VMA intein (6). Furthermore, it suggests that block H is not essential for protein splicing and may be involved in endonuclease activity.

The *Mxe gyrA* deletion did not result from the simple removal of a central region, as indicated by the presence of an uncharacterized linker sequence, and an island motif common to other mycobacterial GyrA inteins (Fig. 2). The presence of this conserved GyrA intein sequence with deletions on either side suggests that this sequence is important for splicing activity or for the unusual process that resulted in these deletions and additions. The C114R mutation is located within the unique *M. xenopi* GyrA linker where it may cause a rearrangement in a predicted β-sheet. The failure of this mutant to splice further indicates the tight structural requirements needed to assure coordinated nucleophilic displacements by the correctly aligned splice junctions and the relevance of distant residues in stabilizing intermediates. Additional mutation and deletion analysis of the linker and island motifs of the *Mxe* GyrA intein may help to elucidate some of these structural issues.

Analysis of the *Mxe* GyrA intein also highlights the importance of certain conditions required to assure splicing or cleavage: (i) the extein sequences in heterologous constructs and (ii) the temperature for splicing and induction of protein expression. Although the intein plus the first C-extein amino acids contain sufficient information for splicing in foreign contexts (3, 14, 15), inteins have most probably coevolved with their cognate exteins to optimize the coordination of the four nucleophilic displacements required to complete the splicing reaction (22). The available evidence suggests that in most cases, protein splicing is a self-catalytic event that does not require the involvement of accessory molecules (4, 7, 9–11, 14, 21, 22). However, the structure of the proximal extein may have relevance, since the extein sequences are immediately adjacent to the splicing active-site residues. In our analysis of the *Mxe* GyrA intein, splicing did not take place efficiently until constructs carrying portions of the native N-extein were tested. The selectivity of this phenomenon is illustrated by the observation that the *M. tuberculosis* RecA intein spliced efficiently in the pYUB53 system, while the equivalent *Mxe* GyrA intein failed to do so. Also, splicing of the *Pyrococcus* sp. GB-D Pol intein-1 in the MIP context does not require polymerase extein sequences (21, 22); however, the *Thermococcus litoralis* Pol intein-2 inserted into the *Eco*RV site of beta-galactosidase requires native extein sequences for efficient splicing (23) and the full-length (420-aa) *M. flavescens* GyrA intein also needs native N-extein sequences for splicing in the context of MIP (14a). Thus, the requirement for native extein sequences is not unique to *M. xenopi* and most likely reflects incompatible extein sequences at the splice junction.

Induction of protein expression at 16°C was also critical for achieving splicing or efficient cleavage in several of the constructs. Failure to splice at 37°C was not due to the generation of insoluble aggregates, but rather from misfolding. Similarly, in the C114R mutant, which is mostly proteolyzed in vivo, the presence of the native C-extein and induction at 16°C stabilized the precursor and resulted in C-terminal cleavage and activation of the N-terminal splice junction (DTT induced N-terminal cleavage). Thus, the functionally competent minimal *Mxe* GyrA intein and its mutants should be attractive candidates for future structural work, particularly in the context of the recently described crystal structure of a topoisomerase II (2), which is a homolog of the mycobacterial GyrA exteins.

What can be the contribution of the *Mxe* GyrA intein to the understanding of the evolution and lateral transmission of splicing elements? Upon demonstration of the independent folding of the intein and endonuclease motifs of the VMA1 intein, Duan et al. (6) hypothesized that these elements represent composite genes resulting from the invasion of an endonuclease open reading frame into a preexisting gene that encoded a protein splicing element. To reconcile this model with the structure of the central region of the *Mxe* GyrA intein, it is necessary to speculate (i) that the *Mxe* element is the only GyrA intein that has not been invaded by an endonuclease open reading frame and that invasion would result in considerable rearrangement or (ii) that the complex deletion-insertion rearrangement occurred after endonuclease acquisition by an ancestral intein, which is more likely.

The small intein of *M. xenopi* also serves to focus discussion on the issue of lateral transmission of inteins. This event has been postulated to occur within the *M. kansasii*, *M. gordonae*, and *M. flavescens* species, as some strains of each species have inteins in their *gyrA* alleles, while others do not (7). This analysis may however be confounded by the fact all three species represent heterogeneous groupings of related organisms sharing phenotypic characters and placed under "convenience" denominations (1, 12, 20). In the case of *M. kansasii*, five genetically distinct groups have been identified (1), only three of which carry GyrA inteins. Within each genetic group, the presence or absence of the intein remains a constant character (1, 18). The GyrA intein of *M. xenopi*, which is also a constant character of the species, lacks homing endonuclease motifs and, therefore, the possibility of invasion of intein-less alleles unless an exogenous homing endonuclease is provided (16a). Taken together, these observations may argue against recent acquisition by lateral transmission of GyrA intein elements in mycobacteria. Future work on evolutionary and structural issues should consider the characteristics of the minimal intein of *M. xenopi*, a paradigm of protein splicing.

## ACKNOWLEDGMENTS

We thank S. Pietrokovski for useful comments and assistance with sequence analysis and S. T. Cole for primers H49 and H50. We thank D. G. Comb for support and encouragement (F.B.P. and M.S.).

This work was funded in part by grants from the Swiss National Science Foundation (grant 31-047.215.96 to A.T.) and United States Public Health Service (grant AI-26170 to W.R.J.) and by a German AIDS-Stipendienprogramm postdoctoral fellowship (S.D.).

## REFERENCES


1. **Alcaide, F., I. Richter, C. Bernasconi, B. Springer, C. Hagenau, R. Schulze-Röbbecke, E. Tortoli, R. Martin, E. C. Böttger, and A. Telenti.** 1997. Heterogeneity and clonality in *Mycobacterium kansasii*: implications for epidemiological and pathogenicity studies. J. Clin. Microbiol. **35:**1959–1964.
2. **Berger, J. M., S. J. Gamblin, S. C. Harrison, and J. C. Wang.** 1996. Structure and mechanism of DNA topoisomerase II. Nature **379:**225–232.
3. **Colston, M. J., and E. O. Davis.** 1994. The ins and outs of protein splicing elements. Mol. Microbiol. **12:**359–363.
4. **Davis, E. O., P. J. Jenner, P. C. Brooks, M. J. Colston, and S. G. Sedgwick.** 1992. Protein splicing in the maturation of *M. tuberculosis* RecA protein: a mechanism for tolerating a novel class of intervening sequence. Cell **71:**201–210.
5. **Doolittle, R. F.** 1993. The comings and goings of homing endonucleases and mobile introns. Proc. Natl. Acad. Sci. USA **90:**5379–5381.
6. **Duan, X., F. S. Gimble, and F. A. Quiocho.** 1997. Crystal structure of the PI-SceI, a homing endonuclease with protein splicing activity. Cell **89:**555–564.
7. **Fsihi, H., V. Vincent, and S. T. Cole.** 1996. Homing events in the *gyrA* gene of some mycobacteria. Proc. Natl. Acad. Sci. USA **93:**3410–3415.
8. **Gimble, F. S., and J. Thorner.** 1992. Homing of a DNA endonuclease gene by meiotic gene conversion in *Saccharomyces cerevisiae*. Nature **357:**301–306.
9. **Hirata, R., Y. Ohsumi, A. Nakano, H. Kawasaki, K. Suzuki, and Y. Araku.** 1990. Molecular structure of a gene, VMA1, encoding the catalytic subunit of $H^+$-translocating adenosine triphosphate from vacuolar membranes of *Saccharomyces cerevisiae*. J. Biol. Chem. **265:**6726–6733.
10. **Hodges, R. A., F. B. Perler, C. J. Noren, and W. E. Jack.** 1992. Protein splicing removes intervening sequences in an archaea DNA polymerase. Nucleic Acids Res. **20:**6153–6157.
11. **Kane, P.-M., C. T. Yamashiro, D. F. Wolczyk, N. Neff, M. Goebl, and T. H. Stevens.** 1990. Protein splicing converts the yeast TFP1 gene product to the 69-kD subunit of the vacuolar $H^+$-adenosine triphosphatase. Science **250:**651–657.
12. **Kirschner, P., and E. C. Böttger.** 1992. Microheterogeneity within rRNA of *Mycobacterium gordonae*. J. Clin. Microbiol. **30:**1049–1050.
13. **Mueller, J. E., M. Bryh, N. Loizos, and M. Belfort.** 1994. Homing endonucleases, p. 111–143. *In* S. M. Linn, R. S. Lloyd, and R. J. Roberts (ed.), Nucleases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
14. **Perler, F. B., G. J. Olsen, and E. Adam.** 1997. Compilation and analysis of intein sequences. Nucleic Acids Res. **25:**1087–1093.

14a. **Perler, F. B.** Unpublished data.

15. **Pietrokovski, S.** 1994. Conserved features of inteins (protein introns) and their use identifying new inteins and related proteins. Protein Sci. **3:**2324–2350.
16. **Pietrokovski, S.** 1996. A new intein in cyanobacteria and its significance for the spread of inteins. Trends Genet **12:**287–288.

16a. **Pietrokovski, S.** Personal communication.

17. **Reith, M., and J. Munholland.** 1995. Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. Plant Mol. Biol. Rep. **13:**333–335.
18. **Sander, P., F. Alcaide, I. Richter, K. Frischorn, E. Tortoli, A. Telenti, and E. C. Böttger.** Inteins in mycobacterial *gyrA* are a phylogenetic character. Microbiology, in press.
19. **Snapper, S. B., R. E. Melton, S. Mustafa, T. Kieser, and W. R. Jacobs, Jr.** 1990. Isolation and characterization of efficient transformation mutants of *Mycobacterium smegmatis*. Mol. Microbiol. **4:**1911–1919.
20. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
21. **Xu, M., M. W. Southworth, F. B. Mersha, L. J. Hornstra, and F. B. Perler.** 1993. In vitro protein splicing of purified precursor and the identification of a branched intermediate. Cell **75:**1371–1377.
22. **Xu, M., and F. B. Perler.** 1996. The mechanism of protein splicing and its modulation by mutation. EMBO J. **15:**5146–5153.

23. **Xu, M., and F. B. Perler.** Unpublished data.