A group I intron in the terminase gene of *Lactobacillus delbrueckii* subsp. *lactis* phage LL-H

Merja Mikkonen and Tapani Alatossava

Author for correspondence: Merja Mikkonen. Tel: +358 81 553 1792. Fax: +358 81 553 1799.
e-mail: Merja.Mikkonen@csc.fi

Department of Genetics,
University of Oulu, FIN-
90570 Oulu, Finland

An 837 nt long group IA intron was discovered in the *Lactobacillus delbrueckii* subsp. *lactis* virulent phage LL-H genome. The LL-H intron conforms well to the secondary structure that is common to all group I introns. The only exception is that the extreme 3' nucleotide of the intron is an A residue instead of the usual G; despite this the intron is efficiently spliced *in vivo*. This LL-H intron contains an ORF, ORF168, which shows homology with endonucleases encoded by ORFs contained in *Bacillus subtilis* phage introns. At present, the LL-H intron is the only one found in the phages of lactic acid bacteria and the first one to be found in a phage belonging to the most abundant taxonomic group, group B or Siphoviridae. The LL-H intron interrupts gene *terL*, the product of which (50.5 kDa, TerL) is significantly homologous to the large subunit of *B. subtilis* phage SPP1 terminase. The product of the upstream gene, *terS* of LL-H (15.9 kDa, TerS), shows homology to small subunits of *B. subtilis* phage terminases.

Keywords: group I intron, RNA structure, terminase, endonuclease, lactic acid bacteria, phage evolution

INTRODUCTION

Interrupted genes are found in all classes of organisms (Lewin, 1994). Most genes in higher eukaryotic genomes are interrupted, but in lower eukaryotes and especially in prokaryotes only a minority contains introns (Lewin, 1994). Introns can be divided into three general classes: nuclear introns, group I introns and group II introns (Lewin, 1994). Group I introns have a wide phylogenetic distribution (Lambowitz & Belfort, 1993). The majority of them have been discovered in fungal mitochondrial DNAs (Cech, 1988; Michel & Westhof, 1990). They also occur in plant mitochondria and chloroplasts, ciliate, slime mould, algal and fungal nuclei, and eubacterial and phage genomes (Cech, 1988; Michel & Westhof, 1990; Lambowitz & Belfort, 1993). Three introns have been found in the *Escherichia coli* phage T4 (Chu *et al.*, 1984; Gott *et al.*, 1986) and one in each of the following *Bacillus subtilis* phages SP01, (Goodrich-Blair *et al.*, 1990) SP82, 2C, Øe (Goodrich-Blair & Shub, 1994) and β22 (Bechhofer *et al.*, 1994). Because of the small number of

characterized introns in eubacteria and their phages, it is impossible to draw firm conclusions about their origin and distribution. In addition to phage introns, only a few examples of group I introns have been discovered in the tRNA genes in cyano- and proteobacteria ('purple bacteria') (Kuhnel *et al.*, 1990; Xu *et al.*, 1990; Reinhold-Hurek & Shub, 1992; Biniszkievich *et al.*, 1994). For this reason, any new eubacterial group I intron will help to understand the role, distribution and origin of these genetic elements.

Group I introns catalyse their own splicing from mRNA via a series of transesterification reactions that are initiated by exogenous guanosine or one of its phosphorylated forms (Cech, 1990). Group I introns don't generally share extensive sequence homology with each other, but they all have a common highly conserved secondary and tertiary structure (Cech, 1988; Michel & Westhof, 1990). The secondary structure contains both local and long-range pairing regions that generate short helices, and highly conserved sequence elements at defined locations (Cech, 1988; Michel & Westhof, 1990). Part of the secondary structure, together with other conserved nucleotides, fold into tertiary structure, forming the catalytic core of the intron, which is crucial for self-splicing (Cech, 1988, 1990; Michel & Westhof, 1990).

The GenBank accession number for the sequence reported in this paper is L37351 (Ilhori168a).

The size of group I introns can vary from about 200 to 3000 nt, mainly depending on whether or not they contain ORFs (Lambowitz & Belfort, 1993). Some of the ORFs overlap core structure elements, but most are located in the peripheral loops (Lambowitz & Belfort, 1993). All but one of the phage introns contain an ORF that is mostly looped out of the core structure (Chu *et al.*, 1984; Gott *et al.*, 1986; Goodrich-Blair *et al.*, 1990; Lambowitz & Belfort, 1993; Bechhofer *et al.*, 1994). Most of the intron-encoded proteins function either as maturases that promote the splicing of their host intron, or act as site-specific endonucleases that make the intron mobile (Lambowitz & Belfort, 1993).

Lactic acid bacteria, especially *Lactobacillus* and *Lactococcus* species, are widely employed in various food and feed industries and biotechnology processes. Lactic acid bacteria are Gram-positive, and belong more precisely to the phylogenetic group of low G + C-content Gram-positives to which *B. subtilis* also belongs (Olsen *et al.*, 1994). Phylogenetically, lactic acid bacteria are quite distant from the Gram-negative *E. coli* (Olsen *et al.*, 1994).

Phage LL-H is a virulent phage of *Lactobacillus delbrueckii* subsp. *lactis* that was first isolated in a Finnish dairy plant in 1972 (Alatossava & Pyhtilä, 1980). With its small isometric head and long non-contractile tail, LL-H is a typical group B1 phage (Forsman & Alatossava, 1991). LL-H is the most studied representative of the DNA homology group, which contains most of the known *Lactobacillus* phages, both virulent and temperate (Mata *et al.*, 1986; Sechaud *et al.*, 1988). The LL-H genome is circularly permuted and terminally redundant double-stranded DNA (34.6 kb) (Forsman & Alatossava, 1991; Trautwetter *et al.*, 1986). So far about 60% of the LL-H genome sequence has been published (Vasala *et al.*, 1993; Mikkonen & Alatossava, 1994; GenBank M96254).

In this paper we describe a group I intron present in the terminase gene of phage LL-H of *Lb. delbrueckii* subsp. *lactis*. This is the first time an intron has been discovered in a phage belonging to the taxonomic group B (or Siphoviridae), the most abundant phage group (Ackermann & DuBow, 1987).

METHODS

Bacteria and phages. *Lb. delbrueckii* subsp. *lactis* LKT was used as the propagation strain for phage LL-H. It was grown at 37 °C in MRS broth (Difco) that was supplemented with 20 mM CaCl₂. Phage propagation, concentration and purification were carried out as described previously (Forsman & Alatossava, 1991). *E. coli* JM101 was grown in 2 × TY broth and used as the host for M13 cloning vectors (Sambrook *et al.*, 1989).

Isolation and manipulation of phage LL-H DNA. Phage particles were purified in a CsCl gradient as described by Alatossava & Pyhtilä (1980). Phage and vector DNA extractions, restriction endonuclease digestions, agarose gel electrophoresis, DNA ligations and *E. coli* transformations were performed by standard methods (Sambrook *et al.*, 1989).

Phage LL-H RNA extraction. Total RNA was isolated from phage LL-H-infected *Lb. delbrueckii* subsp. *lactis* LKT cells

60 min after infection and from non-infected LKT cells. Rifampicin (200 µg ml⁻¹) was used to inhibit RNA synthesis, and cells were collected by centrifugation. The cell pellet was suspended in 100 µl lysis buffer [10 mM MgCl₂, 250 mM NaCl, 50 mM sodium phosphate buffer (pH 5.5), 10% glucose (w/v)] and 5 µl (10 U µl⁻¹) purified LL-H lysis enzyme (kindly provided by Antti Vasala & Merja Välikilä, University of Oulu, Finland) was added. The samples were incubated for 10 min at room temperature, after which the total RNA was purified with the modified detergent/phenol method (Slater, 1984; Vasala *et al.*, 1993).

cDNA synthesis and PCR-amplification. Total RNA isolated from LKT cells was used as a template to synthesize cDNA with 1 µl primer A (10 mM)(5'-ATGTCTAACTGCTGCTT-3') using SuperScript Preamplification System (Gibco BRL) with modifications described by Beresford *et al.* (1993). The cDNA obtained with SuperScript reverse transcriptase was enzymically amplified by PCR and a GeneAmp-kit from Perkin Elmer Cetus, used as specified by the manufacturer. For amplification 5 µl primer A (10 mM) and 5 µl primer B (10 mM)(5'-AGCTGG-TCTTTGACATG-3') were used. The amplification procedure used 30 cycles of denaturation at 92 °C for 1 min, annealing at 46 °C for 1 min and extension at 72 °C for 2 min. The programme included a pre-incubation at 92 °C for 2 min before the first cycle; and after the last cycle an incubation at 72 °C for 10 min that was followed by cooling to 4 °C, which was also the storing temperature. Similarly, 200 ng phage LL-H DNA was amplified with the same protocol and primers.

DNA sequencing. The nucleotide sequence of phage LL-H was determined independently in both directions by the method of Sanger *et al.* (1977). Phage LL-H DNA was sequenced from cloned fragments with Sequenase version 2.0 (USB). The cycle sequencing technique modified from the method of Murray (1989) and TAQuence version 2.0 (USB) were used to sequence the PCR-amplified cDNA and to confirm the LL-H nucleotide sequence with direct sequencing from the phage LL-H DNA. The sequencing reaction products were stored at 4 °C. They were separated on a standard 6% (w/v) acrylamide/7 M urea gel (Sambrook *et al.*, 1989).

Sequence analysis. Computer analyses were performed with the Genetics Computer Group (GCG) sequence analysis software package version 8-UNIX (Devereux *et al.*, 1984), LKB DNASIS (V7.0) and LKB PROSIS (V6.02). Databases were searched with the nucleotide sequence and for the ORFs with both the nucleotide and the amino acid sequences. Database searches were performed with programs FASTA (Pearson & Lipman 1988) and BLAST (Altschul *et al.*, 1990).

RESULTS AND DISCUSSION

The phage LL-H genome contains a group IA intron that is efficiently spliced *in vivo*

During the phage LL-H genome sequencing project the ORF168 was found to share significant amino acid homology with several intron-encoded ORFs of *B. subtilis* phages (Goodrich-Blair *et al.*, 1990; Goodrich-Blair & Shub, 1994). Analysis of the nucleotide sequence confirmed that the phage LL-H genome could contain a group I intron. The ORF168 and the putative intron were located in a 3.1 kb *Pst*I-*Sal*I fragment (Figs 1 and 2; GenBank L37351) that lies between the *pac* site (Forsman & Alatossava, 1991) and the gene *g57* that encodes a minor structural protein gp61 of LL-H (GenBank

Fig. 1. Organization of the phage LL-H genome region containing the LL-H intron and terminase genes. In the phage LL-H restriction map (Forsman & Alatossava, 1991) the genome is shown linearized at its packaging site (*pac*). The previously characterized structural protein genes (*g57*, *g34*, *g17*, *g70*, *g88*, *g71*; Vasala *et al.*, 1993; Mikkonen & Alatossava, 1994) and the lysin gene (*lysA*) of phage LL-H (M96254) are marked with arrows beneath the map. The 3.1 kb *Sall*–*PstI* fragment is shown in more detail below the map. The horizontal arrows show the orientation, position and size of putative genes as derived from the nucleotide sequence presented in this study. At the beginning of an arrow, an open circle denotes an RBS (Mikkonen *et al.*, 1994). The vertical arrowheads indicate the intron splice sites. P, *PstI*; S, *Sall*.

L29567; Mikkonen & Alatossava, 1994). *ORF168* and the putative LL-H intron were located in the main coding strand, which also contains the previously characterized structural-protein-encoding genes (Mikkonen & Alatossava, 1994).

To confirm the position and size of the putative LL-H intron and to test its ability to splice *in vivo*, cDNA was synthesized from LL-H mRNA followed by PCR amplification, and PCR amplification of LL-H genomic DNA that contained the intron. Two primers were used: primer A, 236 nt downstream of the proposed intron, and primer B, 253 nt upstream of the intron. When they were used to amplify genomic LL-H DNA, a 1.35 kb DNA fragment was produced. When the same reaction was performed, but using as the template the cDNA synthesized from LL-H mRNA with reverse transcriptase, a 0.5 kb fragment was produced (results not shown). The observed fragment sizes agreed very well with those calculated from the nucleotide sequence of phage LL-H (Fig. 2). The expected size was 1352 bp for an amplification product from the genomic LL-H DNA, and 515 bp for the cDNA produced from mRNA template after the intron sequence had been removed.

To determine the 5' and 3' splicing sites of the LL-H intron, the PCR-amplified cDNA, produced with reverse transcriptase from LL-H mRNA extracted after *in vivo* intron removal and exon ligation, was sequenced. The sequencing reactions were performed for both strands using primers A and B. With both primers, a sequence was obtained that was identical to the LL-H genomic sequence, but 837 nt had been removed from the ligated exons, joining residues 1423 and 2261 (Figs 2 and 3).

The secondary structure predicted for the phage LL-H

intron conforms well to that of all group I introns (Burke *et al.*, 1987) and is shown in Fig. 3. The secondary structure forms the intron core and is essential for the splicing (Cech, 1990; Michel & Westhof, 1990). It consists of pairing elements from P1 to P10 and of four conserved sequence elements that always occur in the same order, 5'-PQRS-3' (Burke *et al.*, 1987; Cech, 1988). In the LL-H intron, the sequences of these elements match the consensus well (Cech, 1988). All conserved pairing elements are present in the LL-H intron, except that it does not contain the P2 element, which is found in only about two-thirds of group I introns (Michel & Westhof, 1990). For example, the T4 phage intron in the gene *sunY* has no P2 (Shub *et al.*, 1988).

Between stems P7 and P3 of the LL-H intron, two extra stem-loop structures, P7.1 and P7.2, could be predicted (Fig. 3). This feature is characteristic for introns belonging to the various IA subgroups (Cech, 1988; Michel & Westhof, 1990). In subgroups IA2 and IA3, there are two stem-loops but in IA1 there is only one (Michel & Westhof, 1990). Based on this, the LL-H intron seems to belong to subgroup IA2 or IA3 (Michel & Westhof, 1990). The other phage introns so far described belong to subgroup IA2 (Michel & Westhof, 1990; Bechhofer *et al.*, 1994; Goodrich-Blair & Shub, 1994).

The 5' splicing site of group I introns lies in the pairing region P1 formed by the 3' end of the 5' exon and the 5' end of the intron sequence (Cech, 1988, 1990; Michel & Westhof, 1990). The splicing site is nearly always preceded by a U that pairs with a G (Cech, 1988, 1990; Michel & Westhof, 1990). The exon–intron boundary of the LL-H intron was located before the U of the last U–G pair (nt 1424 and 1432 in Fig. 3).

Fig. 2. Nucleotide sequence of the 3116 bp *Pst*I-*Sa*I fragment (this study, GenBank L37351) and 96 bp from the 5' end of genome region encoding structural proteins of phage LL-H (Mikkonen & Alatossava, 1994). It contains the LL-H intron and the putative terminase genes *terS* and *terL*, which consists of two exons *terL1* and *terL2*. The proteins encoded by the LL-H sequence are marked beneath the nucleotide sequence in standard one letter amino acid code. Aligned to the amino acid sequences are examples of homologous amino acid sequences found in databases. Gaps introduced to

Fig. 3. Proposed secondary structure of the LL-H intron. Arrows indicate the 5' and 3' splice-sites, determined by sequencing of the PCR-amplified cDNA synthesized from mRNA. Lowercase letters denote the exon sequences and the capital letters denote the intron sequence. Conserved secondary structure elements of group I introns are labelled with numbered circles, also marked are the conserved sequence elements P, Q, R and S (Burke *et al.*, 1987; Cech, 1988). Lines have been added to make the figure less crowded; no nucleotides have been omitted except where specifically marked (loops L6b and L7.1). The start codon (AUG) of *ORF168* is marked in the L6b and the stop codon (UAA) is boxed. The formation of the P9.0 and P10 is shown in the insert in the right lower corner (the situation depicted is just before the ligation step). The secondary structure was determined manually with the help of GCG programs RNAFOLD, SQUIGGLS, STEMLOOP and REPEAT. The numbering of nucleotides is the same as in Fig. 2.

It was evident from the sequence of the ligated exons that the 3' splicing site of the LL-H intron was located before nt 2261 (Figs 2 and 3). The extreme 3' nucleotide of the LL-H intron was an A, which was confirmed by direct PCR sequencing from the LL-H genomic DNA. In all the previously reported group I introns, the nucleotide preceding the 3' splice site has always been a G residue (Cech, 1988; Michel & Westhof, 1990). However, it has been shown by mutational analysis of the *Tetrahymena thermophila* intron that if the 3' terminal G residue has been changed into an A, the 3' splicing is slightly delayed but still remains accurate (Michel *et al.*, 1989). The four intron nucleotides (3'-GCUU-5') immediately preceding the G

residue pairing with the last U of the 5' exon can pair with the first four nucleotides of the 3' exon (5'-CGAA-3') forming P10 of LL-H intron (Fig. 3). The intron sequences of P1 and P10 form the internal guide sequence that pairs with the exons and aligns them for ligation (Michel *et al.*, 1989). In the LL-H intron P9.0 could be formed by two A-U pairs (nt 2205–2206 and 2258–2259 in Fig. 3). Most often the P9.0 pairing contains two base pairs, though it is not present in all group I introns (Burke, 1989; Michel *et al.*, 1989; Michel & Westhof, 1990). If neither P9.0 nor P10 is present, the 3' splicing is disrupted, but the presence of even one of them restores it (Michel *et al.*, 1989). Because the LL-H intron contains

optimize the alignments are indicated by dots in the nucleotide sequence and by dashes in the amino acid sequence. Identical amino acids are in boxed capital letters and functionally similar amino acids are in capital letters. The putative amino acid sequence motifs are underlined and marked in figure. Termination codons are marked with asterisks. Vertical lines indicate the 5' and 3' splice-sites of the 837 nt intron. The putative RBSs are underlined in the nucleotide sequence and the *Pst*I and *Sal*I recognition sites are boxed. The division of amino acids into groups of functional similarity was: 1, small, non-aliphatic, non-charged (Ala, Ser, Thr, Pro, Gly); 2, polar, not-positively charged (Asn, Asp, Glu, Gln); 3, positively charged (His, Arg, Lys); 4, aliphatic and methionine (Met, Leu, Ile, Val); 5, aromatic, non-charged (Phe, Tyr, Trp) (LKB PROSIS, V6.02).

both the P10 and P9.0 pairings, it may help the LL-H intron to splice correctly and efficiently *in vivo*, as shown above by PCR amplification and sequencing, even though the 3' terminal residue of the LL-H intron is an A instead of the common G.

The LL-H intron contains an ORF of 504 nt that potentially encodes a protein of 168 amino acids (Fig. 2), the calculated molecular mass of which is 20.0 kDa. *ORF168* mostly lies outside the secondary structure essential for group I intron splicing. It begins in the large peripheral loop of P6 (L6b) and its 3' end forms the 3' side of stem P6 and the stop codon is located in the very conserved joining section J6/7 (Fig. 3). The initiation codon of *ORF168* and its putative RBS (Mikkonen *et al.*, 1994) are located in a region separate from the secondary structure required for splicing (Fig. 3).

All but one of the previously described phage introns contain an ORF that is typically located in one of the peripheral loops (Shub *et al.*, 1988; Goodrich-Blair *et al.*, 1990; Goodrich-Blair & Shub, 1994; Bechhofer *et al.*, 1994). The amino acid sequence translated from LL-H *ORF168* was used as a template to search through various databases. Statistically significant amino acid alignments were obtained with the intron-encoded ORFs from the *B. subtilis* phages SP01, SP82, 2C and Øe (Goodrich-Blair *et al.*, 1990; Goodrich-Blair & Shub, 1994), and from a free-standing ORF of *B. subtilis* phage SPP1 (Pedre *et al.*, 1994). All homologies obtained to the *ORF168* product were quite similar and concentrated on the amino-terminal part of each protein (e.g. SP01, 42.1% identity and 61.1% similarity in the first 95 amino acids; Fig. 2). The intron-encoded proteins of the *B. subtilis* phages SP01 and SP82, are known to be site-specific DNA endonucleases (Goodrich-Blair & Shub, 1994). The intron-encoded endonucleases contain a recently described HI-NL-H motif (Goodrich-Blair & Shub, 1994; Shub *et al.* 1994), which also was found in the product of *ORF168* (Fig. 2). The same motif is found in a bacterial restriction endonuclease and in the Zn-finger-like domains of several group II intron ORFs (Goodrich-Blair & Shub, 1994; Shub *et al.* 1994). The similarity of the LL-H intron-encoded *ORF168* to the *B. subtilis* phage intron-ORFs suggests that its product is also an endonuclease. The high similarity of the amino acid sequences in their amino-terminal part may reflect structures that are needed for the formation of the active site of the endonuclease, while the highly variable carboxyl termini could be involved in DNA target site recognition (Goodrich-Blair & Shub, 1994).

Phage LL-H belongs to the taxonomic group B, Siphoviridae, phages with long non-contractile tails (Ackermann & DuBow, 1987; Forsman & Alatossava, 1991). The Siphoviridae family is the most abundant of phage groups; over 50% of all phages reported up to 1987 belong to this group (Ackermann & DuBow, 1987). All the previously reported group I introns have been found in phages belonging to taxonomic group A, Myoviridae, phages with contractile tails (Ackermann & DuBow, 1987), and having large and complex genomes (Chu *et al.*,

1984; Gott *et al.*, 1986; Ackermann & DuBow, 1987; Mosig & Eiserling, 1988; Stewart, 1988; Goodrich-Blair *et al.*, 1990; Goodrich-Blair & Shub, 1994; Bechhofer *et al.*, 1994). Compared to these group A phages, LL-H has a much smaller genome, 34.6 kb (Forsman & Alatossava, 1991), being only about one-quarter of that of the previously described intron-containing phages. With the present discovery of the LL-H intron, group I introns have now been found in the two distinct phage taxonomic groups A and B, to which about 80% of all known phages belong (Ackermann & DuBow, 1987). These discoveries could mean that bacterial introns might be much more common than previously suspected.

The LL-H intron interrupts a terminase gene

To understand the possible biological role of the LL-H intron one should know about the interrupted gene and how intron splicing could affect its function. The nucleotide sequence of the 3.1 kb *Pst*I–*Sal*I fragment containing the LL-H intron was analysed for possible coding regions. In the main coding strand three coding regions, two upstream of and one downstream of the LL-H intron, were detected (Figs 1 and 2). The amino acid sequences translated from the three coding regions (*terS*, *terL1* and *terL2* in Figs 1 and 2) were used as templates in database searches. Statistically significant amino acid alignments were obtained with terminase enzyme subunits of *B. subtilis* phages SPP1, SF6 and ρ15 (Chai *et al.*, 1992, 1994). Like LL-H they are *pac*-type phages, and the products of *g1* (gp1) and *g2* (gp2), encoding the small and large subunits of the SPP1-family of terminases (Chai *et al.*, 1992, 1994), are essential for the *pac*-site cleavage. The small terminase subunits (gp1) of all three *B. subtilis* phages were about equally homologous to TerS of phage LL-H. The large terminase subunit (gp2) has been characterized only from phage SPP1 (Chai *et al.*, 1992). The amino-terminal part of TerS is 43.8% identical and 62.0% similar to the first 121 amino acids of gp1 of SPP1. The TerL1 is 30.4% identical and 55.1% similar to the 133 amino-terminal amino acids of gp2 of SPP1 and TerL2 is 23.8% identical and 45.0% similar to the 289 carboxy-terminal amino acids of gp2 of SPP1. *terS* potentially encodes a protein of 142 amino acids, the calculated molecular mass of which is 15.9 kDa. Removal of the LL-H intron and exon (*terL1* and *terL2*) ligation form the combined ORF *terL*, which potentially encodes the TerL protein of 433 amino acids with calculated molecular mass 50.5 kDa. Additional evidence for the biological activity of the LL-H intron is that the formation of TerL, homologous counterpart of gp2 of phage SPP1, is possible only after the LL-H intron self-splices *terL* mRNA.

Phage terminase genes characteristically overlap by a few nucleotides and the DNA interaction sites are either within, or close to and upstream of, genes encoding the terminase proteins (Black, 1989; Chai *et al.*, 1992; Powell *et al.*, 1990; Feiss, 1986). Phage LL-H terminase genes *terS* and *terL* overlap by eight nucleotides and the LL-H *pac* site has been located about 1.2 kb upstream from *terS*

(Forsman & Alatossava, 1991). Phage terminases are hetero-oligomers consisting of small and large subunits (Black, 1989). The small subunit is important for the *pac*-site recognition and DNA binding, and it also appears to bind and hydrolyse ATP (Black, 1989). A putative NTP-binding motif (AXXXXGKL, motif A of Walker *et al.*, 1982), DNA binding motif (helix-turn-helix) and NTP hydrolysis motif (DE, motif B of Walker *et al.*, 1982) have been identified in the amino acid sequence of gp1 of phages SPP1, SF6 and $\rho 15$ (Chai *et al.*, 1992, 1994). These motifs can also be found in TerS of phage LL-H (Fig. 2). The large subunit appears to bind to the prohead and may be involved in the cutting of phage DNA molecules (Black, 1989; Feiss, 1986). TerS and TerL could represent the small and large subunits of LL-H terminase, respectively, and belong to the SPP1-family of terminases (Chai *et al.*, 1992, 1994).

ACKNOWLEDGEMENTS

This study was supported by grants from the Ministry of Agriculture, the Academy of Finland (SA 1051317) and CEC BIOTECH program (G-project BIO2-CT94-3055). We thank Anne Karjalainen for excellent technical assistance. The purified LL-H lysin enzyme was kindly provided by our colleagues Antti Vasala Phil. lic. and Merja Valkkil MSc, Department of Genetics, University of Oulu.

REFERENCES

- Ackermann, H.-W. & DuBow, M. S. (1987). *Viruses of Prokaryotes*, vols 1 and 2. Boca Raton: CRC Press.
- Alatossava, T. & Pyhtil, M. J. (1980). Characterization of a new *Lactobacillus lactis* bacteriophage. *IRCS Med Sci* **8**, 297–298.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Bechhofer, D. H., Hue, K. K. & Shub, D. A. (1994). An intron in the thymidylate synthase gene of *Bacillus* bacteriophage $\beta 22$: evidence for independent evolution of a gene, its group I intron, and the intron open reading frame. *Proc Natl Acad Sci USA* **91**, 11669–11673.
- Beresford, T. P. J., Ward, L. J. H. & Jarvis, A. W. (1993). Temporally regulated transcriptional expression of the genomes of lactococcal bacteriophages c2 and sk1. *Appl Environ Microbiol* **59**, 3708–3712.
- Biniszkiewicz, D., Cesnaviciene, E. & Shub, D. A. (1994). Self-splicing group I intron in cyanobacterial initiator methionine tRNA: evidence for lateral transfer of introns in bacteria. *EMBO J* **13**, 4629–4635.
- Black, L. W. (1989). DNA packaging in dsDNA bacteriophages. *Annu Rev Microbiol* **43**, 267–292.
- Burke, J. M. (1988). Molecular genetics of group I introns: RNA structures and protein factors required for splicing – a review. *Gene* **73**, 273–294.
- Burke, J. M., Belfort, M., Cech, T. R., Davies, R. W., Schweyen, R. J., Shub, D. A., Szostak, J. W. & Tabak, H. F. (1987). Structural conventions for group I introns. *Nucleic Acids Res* **15**, 7217–7221.
- Cech, T. R. (1988). Conserved sequences and structures of group I introns: building an active site for RNA catalysis – a review. *Gene* **73**, 259–271.
- Cech, T. R. (1990). Self-splicing of group I introns. *Annu Rev Biochem* **59**, 543–568.
- Chai, S., Bravo, A., Luder, G., Nedlin, A., Trautner, T. A. & Alonso, J. C. (1992). Molecular analysis of *Bacillus subtilis* bacteriophage SPP1 region encompassing genes 1 to 6. *J Mol Biol* **224**, 87–102.
- Chai, S., Kruft, V. & Alonso, J. C. (1994). Analysis of the *Bacillus subtilis* bacteriophage SPP1 and SF6 gene 1 product: a protein involved in the initiation of headful packaging. *Virology* **202**, 930–939.
- Chu, F. K., Maley, G. F., Maley, F. & Belfort, M. (1984). Intervening sequence in the thymidylate synthase gene of bacteriophage T4. *Proc Natl Acad Sci USA* **81**, 3049–3053.
- Devereux, J., Haeblerli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Feiss, M. (1986). Terminase and the recognition, cutting and packaging of λ chromosome. *Trends Genet* **2**, 100–104.
- Forsman, P. & Alatossava, T. (1991). Genetic variation of *Lactobacillus delbrueckii* subsp. *lactis* bacteriophages isolated from cheese processing plants in Finland. *Appl Environ Microbiol* **57**, 1805–1812.
- Goodrich-Blair, H. & Shub, D. A. (1994). The DNA polymerase genes of several HMU-bacteriophages have similar group I introns with highly divergent open reading frames. *Nucleic Acids Res* **22**, 3715–3721.
- Goodrich-Blair, H., Scarlato, V., Gott, J. M., Xu, M.-Q. & Shub, D. A. (1990). A self-splicing group I intron in the DNA polymerase gene of *Bacillus subtilis* bacteriophage SPO1. *Cell* **63**, 417–424.
- Gott, J. M., Shub, D. A. & Belfort, M. (1986). Multiple self-splicing introns in bacteriophage T4: evidence from autocatalytic GTP labeling of RNA *in vitro*. *Cell* **47**, 81–87.
- Kuhse, M. G., Strickland, R. & Palmer, J. D. (1990). An ancient group I intron shared by eubacteria and chloroplasts. *Science* **250**, 1570–1573.
- Lambowitz, A. M. & Belfort, M. (1993). Introns as mobile genetic elements. *Annu Rev Biochem* **62**, 587–622.
- Lewin, B. (1994). *Genes V*. New York: Oxford University Press.
- Mata, M., Trautwetter, A., Luthaud, G. & Ritzenthaler, P. (1986). Thirteen virulent and temperate bacteriophages of *Lactobacillus bulgaricus* and *Lactobacillus lactis* belong to a single DNA homology group. *Appl Environ Microbiol* **52**, 812–818.
- Michel, F. & Westhof, E. (1990). Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. *J Mol Biol* **216**, 585–610.
- Michel, F., Hanna, M., Green, R., Bartel, D. P. & Szostak, J. W. (1989). The guanosine binding site of the *Tetrahymena* ribozyme. *Nature* **342**, 391–395.
- Mikkonen, M. & Alatossava, T. (1994). Characterization of the genome region encoding structural proteins of *Lactobacillus delbrueckii* subsp. *lactis* bacteriophage LL-H. *Gene* **151**, 53–59.
- Mikkonen, M., Vuoristo, J. & Alatossava, T. (1994). Ribosome binding site consensus sequence of *Lactobacillus delbrueckii* subsp. *lactis* bacteriophage LL-H. *FEMS Microbiol Lett* **116**, 315–320.
- Mosig, G. & Eiserling, F. (1988). Phage T4 structure and metabolism. In *The Bacteriophages*, vol. 2, pp. 521–606. Edited by R. Calendar. New York & London: Plenum Press.
- Murray, V. (1989). Improved double-stranded DNA sequencing using the linear polymerase chain reaction. *Nucleic Acids Res* **17**, 8889.
- Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* **85**, 2444–2448.
- Pedre, X., Weise, F., Chai, S., Lueder, G. & Alonso, J. C. (1994). Analysis of cis and trans acting elements required for the initiation

of DNA replication in *Bacillus subtilis* bacteriophage SPP1. *J Mol Biol* **236**, 1324–1340.

Powell, D., Franklin, J., Arisaka, F. & Mosig, G. (1990). Bacteriophage T4 DNA packaging genes 16 and 17. *Nucleic Acids Res* **18**, 4005.

Olsen, G. J., Woese, C. R. & Overbeek, R. (1994). The winds of (evolutionary) change: breathing new life into microbiology. *J Bacteriol* **176**, 1–6.

Reinhold-Hurek, B. & Shub, D. A. (1992). Self-splicing introns in tRNA genes of widely divergent bacteria. *Nature* **357**, 173–176.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.

Sechaud, L., Cluzel, P.-J., Rousseau, M., Baumgartner, A. & Accolas, J.-P. (1988). Bacteriophages of lactobacilli. *Biochimie* **70**, 401–410.

Shub, D. A., Gott, J. M., Xu, M.-Q., Lang, B. F., Michel, F., Tomaschewski, J., Pedersen-Lane, J. & Belfort, M. (1988). Structural conservation among three homologous introns of bacteriophage T4 and the group I introns of eukaryotes. *Proc Natl Acad Sci USA* **85**, 1151–1155.

Shub, D. A., Goodrich-Blair, H. & Eddy, S. R. (1994). Amino acid sequence motif of group I intron endonucleases is conserved in

open reading frames of group II introns. *Trends Biochem Sci* **19**, 402–404.

Slater, R. J. (1984). The extraction of total RNA by the detergent and phenol method. In *Methods in Molecular Biology: Nucleic Acids*, vol. 2, pp. 101–108. Edited by J. M. Walker. Clifton: Humana Press.

Stewart, C. (1988). Bacteriophage SPO1. In *The Bacteriophages*, vol. 1, pp. 477–515. Edited by R. Calendar. New York & London: Plenum Press.

Trautwetter, A., Ritzenthaler, P., Alatossava, T. & Mata-Gilsinger, M. (1986). Physical and genetic characterization of the genome of *Lactobacillus lactis* bacteriophage LL-H. *J Virol* **59**, 551–555.

Vasala, A., Dupont, L., Baumann, M., Ritzenthaler, P. & Alatossava, T. (1993). Molecular comparison of the structural proteins encoding gene clusters of two related *Lactobacillus delbrueckii* bacteriophages. *J Virol* **67**, 3061–3068.

Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982). Distantly related sequences in the α - and β -subunits of ATP synthase myosin, kinase and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J* **1**, 945–951.

Xu, M.-Q., Kathe, S. D., Goodrich-Blair, H., Nierzwicki-Bauer, S. A. & Shub, D. A. (1990). Bacterial origin of a chloroplasts intron: conserved self-splicing group I introns in cyanobacteria. *Science* **250**, 1566–1570.

Received 20 February 1995; revised 15 May 1995; accepted 5 June 1995.