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Characterization of the genes encoding integrative and excisive functions of *Lactobacillus* phage øg1e: cloning, sequence analysis, and expression in *Escherichia coli*

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Abstract

øg1e is a temperate phage of the *Lactobacillus* strain G1e. The phage-host junctions *attR* and *attL* cloned from the lysogen have a 24-bp common (core) sequence implicated in recombination. DNA sequencing analysis of a 5.2-kbp *Sac*I fragment of the øg1e phage genome (42.5 kbp) revealed two possible open reading frames (ORF), *xis* and *int*, and the phage attachment (recombination) site (*attP*), whose 24-bp sequence is identical to the core sequence detected in *attR* and *attL*. The deduced *int* product (Int) is a basic protein of 391 amino acids with an estimated p*I* of 9.70, and significantly resembles other presumed integrases encoded by the *Lactobacillus* and *Lactococcus* phages including øadh and øLC3, as well as the *Escherichia coli* phages such as λ.The predicted øg1e *xis* protein (Xis) is small and very acidic (66 amino acids; p*I* 4.55), and shows a resemblance (32% overall identity) with a putative excisionase encoded by the *Staphylococcus* phage ø11. The øg1e Int with a deduced molecular mass of 45.5 kDa was overproduced in *E. coli* cells, and electrophoretically analyzed.

Keywords: Bacteriophage øg1e; Lactic acid bacteria; Integrase; Excisionase; DNA sequence

1. Introduction

Prevalence of lysogeny in various lactic acid bacteria has been reported (Davidson et al., 1990). The lysogenic phages are thought to encode a set of proteins, which function in recombinational processes of its genome DNA, moving into and out of the host chromosome (integration and excision, respectively).

In coliphages λ and P2 (Leong et al., 1986; Landy, 1989; Campbell, 1994), their integration and excision into and out of the host genome, respectively, depend upon two phage-encoded proteins, besides host proteins such as an integration host factor (IHF). One of these phage proteins, known as an integrase, acts as a site-specific recombinase in both of the integrative and excisional processes, whereas the other protein (an excisionase) participates only in the excision process through a cooperation with integrase.

Several putative integrases have been reported from the *lactobacillus* phage øadh (Fremaux et al., 1993) and mv4 (Dupont et al., 1995), and the *lactococcus* phage øLC3 (Lillehaug and Birkeland, 1993), Tuc2009 (Van de Guchte et al., 1994), and BK5-T (Boyce et al., 1995).

Abbreviations: aa, amino acids; *attB*, bacterial attachment site; *attR* and *attL*, phage-host junctions (right and left, respectively); *attP*, phage attachment site; bp, base pair; CBB, Coomassie Brilliant Blue; *cpg*, gene encoding repressor (Cpg); IHF, integration host factor; *int*, gene encoding integrase (Int); IPTG, isopropyl β-D-thiogalactopyranoside; LB, Luria-Bertani (medium); *Lorf143*, ORF encoding 143-aa protein (gp*Lorf143*); *Lorf304*, ORF encoding 304-aa protein (gp*Lorf304*); M_w, molecular weight; ORF, putative open reading frame; p*I*, isoelectric point; PAGE, polyacrylamide gel electrophoresis; p*lac*, promoter of lacZ′ gene; *RB*, ribosomal binding site; *Rorf148*, ORF encoding 148-aa protein; *Rorf192*, ORF encoding 192-aa protein; *Rorf232*, ORF encoding 232-aa protein; *RS*, repeated sequence; SDS, sodium dodecyl sulfate; *Ti*, possible secondary structure; *xis*, gene encoding excisionase (Xis); YT, yeast-Triptone (medium).

Unlike the lambdoid phages, however, molecular details on integration and excision of the phages of lactic acid bacteria are still insufficient; structures and functions of their integration and excision genes mostly remain to be elucidated.

Recently, we isolated a new *Lactobacillus* temperate phage øg1e, and identified several genes specifying the capsid proteins (Kakikawa et al., 1996), and the cytolytic function (Oki et al., 1996a).

In this report, we describe cloning and sequencing of the phage-host junctions between øg1e and its host G1e, and øg1e genes *xis* and *int* for its integration and/or excision, as well as the phage attachment (recombination) site (*attP*).

2. Materials and methods

2.1. Bacteria, phage and plasmids

The *Lactobacillus* strain G1e and its temperate phage øg1e are from our laboratory stock, and were propagated in M17 broth as described previously (Kakikawa et al., 1996). The *E. coli* vector plasmids (pUC18, pUC19, pUC118 and pUC119) and their host XL1-Blue are from our laboratory stock, and were propagated using LB or 2YT broth (Oki et al., 1996a).

2.2. Analysis of DNA and protein

The øg1e DNA was extracted from the virus particles (Kakikawa et al., 1996). The G1e chromosomal DNA was prepared by the method of Katty et al. (1989). Southern blot hybridization was performed as described previously (Oki et al., 1996b); ^{32}P-labeled probes were prepared by nick translation. Cloning of øg1e DNA was carried out essentially as described by Oki et al. (1996a). Recombinant plasmids were introduced into *E. coli* XL1-Blue cells by Ca^{2+}-dependent transformation or electroporation (Oki et al., 1996b). For DNA sequencing, various deletion clones were constructed from the restriction library of øg1e DNA, by exonuclease digestion (Oki et al., 1996a). The DNA sequence was determined by the chain termination method (Sanger et al., 1982). SDS-polyacrylamide gel electrophoresis (PAGE) was performed as described by Kakikawa et al. (1996): proteins were visualized by staining with Coomassie Brilliant Blue (CBB) R-250.

All other procedures were performed as described previously (Kodaira et al., 1996).

2.3. Enzymes and biochemicals

Restriction enzymes, phage T4 DNA ligase, and alkaline phosphatase (calf intestine) were purchased from

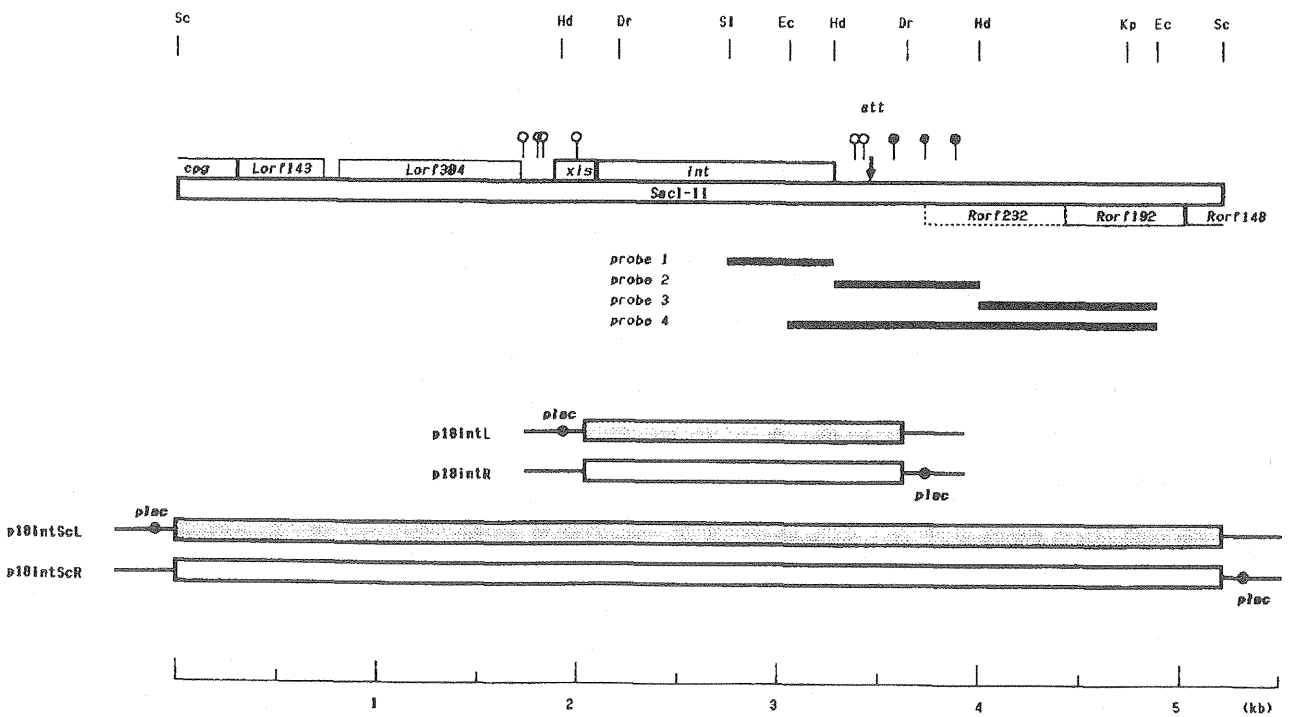

Fig. 1. Physical map of the øg1e *xis-int* region. A 5204-bp *Sac*I fragment (*Sac*I–II) sequenced in this study (see text and Fig. 4) was shown. The possible ORFs were indicated by boxes above the fragment. *Rorf232* has no putative ribosomal binding sites. Dr, *Dra*I; Ec, *Eco*RI; Hd, *Hin*dIII; Kp, *Kpn*I; Sl, *Sal*I. Open circle, inverted repeat; closed circle, ρ-independent-like mRNA terminator. The four thick lines (probes 1–4) indicate restriction fragments used as probes for Southern blot analysis. The four recombinant plasmids constructed in this study (see text) were shown under the probes: dotted boxes, under the *lacZ'* promoter (small closed circle); open boxes, free from the *lacZ'* promoter.

Takara Shuzo (Kyoto) and Nippon Gene (Toyama). Buffers for each enzymes were as recommended by the manufacturers.

[α-^{32}P]dCTP was from NEN (USA). All other materials were as described previously by Kodaira et al. (1996).

3. Results and discussion

3.1. Cloning and sequence of the øgle DNA

As a prophage, øgle genome is probably integrated within the host Gle chromosome between a phage attachment site (*attP*) and a host bacterial attachment site (*attB*), to generate two additional sites at the right (*attR*) and left (*attL*) junctions. For mapping the phage-host junctions, DNAs extracted from øgle and Gle were digested with several restriction enzymes, and subjected to Southern blot analysis with various øgle phage DNA fragments as probes (e.g., probes 1, 2, 3 and 4; see Fig. 1). When probe 2 (a 0.7-kbp *Hin*dIII fragment) was hybridized to *Sac*I digests of the Gle DNA (Fig. 2), two bands of about 4.3 and 24 kb, corresponding to the junction fragments (designated as *attL* and *attR*, respectively) (Fig. 2), were detected, in addition to the 5.2-kb *Sac*I øgle fragment containing

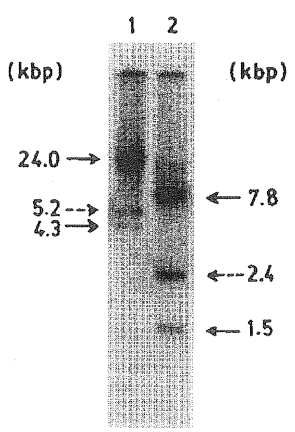

Fig. 2. Detection of the phage-host junctions. DNA extracted from Gle lysogen was digested with a restriction enzyme (*Sac*I and/or *Sal*I), and subjected to Southern blot hybridization (Section 2) probed to a 0.7-kbp *Hin*dIII fragment (probe 2) of øgle phage DNA (see Fig. 1). Lane 1, *Sac*I; lane 2, *Sac*I and *Sal*I (a few partial bands were located above a 7.8-kbp fragment). Arrows indicate the fragments containing the right (*attR*) or left (*attL*) junction (for the details, see text). Dotted arrows represent phage DNA fragments (see text).

attP (see below and also Fig. 1 and Fig. 4). Like the most related *Lactobacillus* phage mv4 (Dupont et al., 1995), an extrachromosomal form of øgle DNA seems to coexist with the integrated prophage form.

The DNA region containing *attL* (1.5-kb *Sac*I/*Sal*I fragment, Fig. 2) or *attR* (0.6-kb *Hha*I fragment, data not shown) was cloned into *E. coli* pUC118, and sequenced. As shown in Fig. 3, both *attR* and *attL* have an identical 24-bp stretch (5′-TGACCCCACCCG-GACTCGAACCGG-3′), that is common to *attP* (see below and Fig. 4). The 24-bp sequence appears to function as a core domain for the strand exchange reaction in the øgle integration and/or excision pathways.

For analysis of *attP* and its surroundings, the 5.2-kbp *Sac*I–II fragment of øgle phage DNA (Fig. 1) was cloned into a *Sac*I site of pUC18, resulting in two recombinants, p18intScL and p18intScR (Fig. 1). As summarized in Fig. 1, sequencing analysis of the 5204-bp *Sac*I–II DNA (Section 2) revealed that one strand contains five possible ORFs in addition to a 24-bp *attP* sequence (see above), whose order is *cpg-Lorf143-Lorf304-xis-int-attP*, whereas the complementary strand (R-strand) has three potential ORFs *Rorf148-Rorf192-Rorf232* (the two putative ORFs *cpg* and *Rorf148* are truncated). These predicted ORFs (except *Rorf232*) are preceded by a possible ribosomal binding sequence (Table 1, see also Fig. 3), which is in good agreement with those of several genes found in *Lactobacillus* and *Lactococcus* species: 5′-AGGAGG-3′ (Pouwels and Leer, 1993) and 5′-AGAAAGGAGGT-3′ (Ludwig et al., 1985; Schouler et al., 1994), respectively.

In Fig. 4, a 2.8-kbp øgle DNA containing *xis-int-attP* was shown together with their deduced amino acid sequences. The predicted *int* product (termed Int) is a basic protein of 391 amino acids (for details, see below), whose estimated molecular mass and p*I* are 43 kDa and 9.70, respectively (Table 1). On the other hand, the putative *xis* product (Xis) (see below) is a small and very acidic protein (66 amino acids; 7.6 kDa; p*I* 4.55) (Table 1). Recently, we have cloned and sequenced *cpg* (Kodaira et al., unpublished results), demonstrating that its putative product of 132 amino acids contains a helix-turn-helix motif in its N-terminus, and resembles other repressors encoded by the lambdoid phages such as λ, P2 and P22 (Ljungquist et al., 1984). The roles of *Lorf143*, *Lorf304* and *Rorf192* (Fig. 1 and Table 1) in the øgle multiplication have not been ascertained. On

```
attL   5'-TACAATAATCAACCGTAAATAACGGTTTGA TGACCCCACCCGGACTCGAACCGG GATCAACCGCTTAGGAGGCGGGTGCCCTAT-3'
attP   5'-TACAATAATCAACCGTAAATAACGGTTTGA TGACCCCACCCGGACTCGAACCGG TGTTCAGAGAGCACTGACATATCAGTGTTT-3'
attR   5'-TATAGGACGGTTTTTGAAGAAATATTTAAC TGACCCCACCCGGACTCGAACCGG TGTTCAGAGAGCACTGACATATCAGTGTTT-3'
```

Fig. 3. DNA sequences of the phage-host junctions. The two phage-host junctions, *attR* and *attL* (see Fig. 2), were cloned (see text), and sequenced. The 24-bp common sequences among *attR*, *attL* and *attP* (see Fig. 4) were boxed.

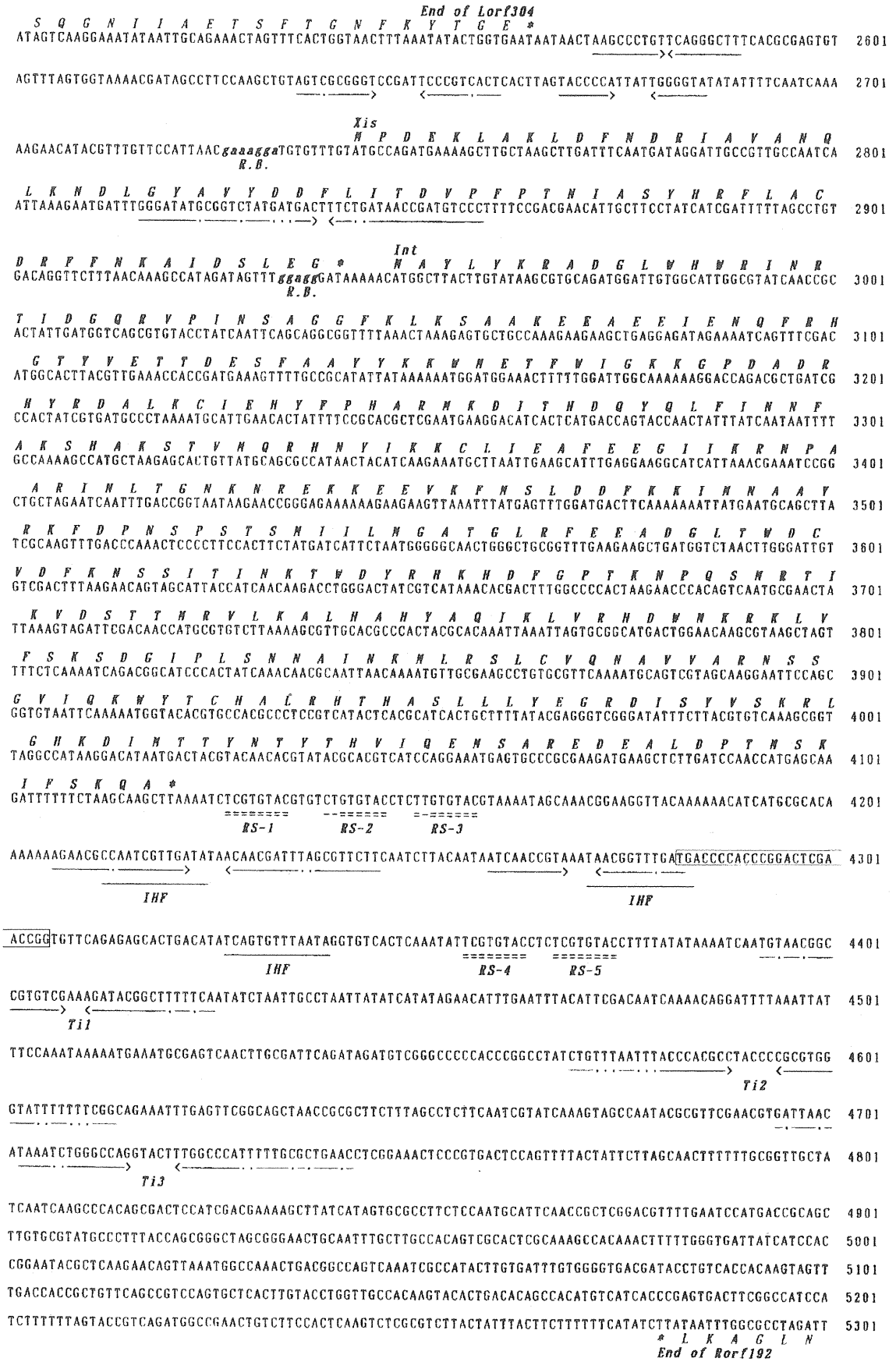
End of Lorf304
S Q G N I I A E T S F T G N F K Y T G E *
ATAGTCAAGGAAATATAATTGCAGAAACTAGTTTCACTGGTAACTTTAAATATACTGGTGAATAATAACTAAGCCCTGTTCAGGGCTTTCACGCGAGTGT 2601
AGTTTAGTGGTAAAACGATAGCCTTCCAAGCTGTAGTCGCGGGTCCGATTCCCGTCACTCACTTAGTACCCCATTATTGGGGTATATATTTTCAATCAAA 2701
Xis
M P D E K L A K L D F N D R I A V A N Q
AAGAACATACGTTTGTTCCATTAACgaaaggaTGTGTTTGTATGCCAGATGAAAAGCTTGCTAAGCTTGATTTCAATGATAGGATTGCCGTTGCCAATCA 2801
R.B.
L K N D L G Y A V Y D D F L I T D V P F P T N I A S Y H R F L A C
ATTAAAGAATGATTTGGGATATGCGGTCTATGATGACTTTCTGATAACCGATGTCCCTTTTCCGACGAACATTGCTTCCTATCATCGATTTTAGCCTGT 2901
Int
D R F F N K A I D S L E G * N A Y L Y K R A D G L W H W R I N R
GACAGGTTCTTTAACAAAGCCATAGATAGTTTggaggGATAAAAACATGGCTTACTTGTATAAGCGTGCAGATGGATTGTGGCATTGGCGTATCAACCGC 3001
R.B.
T I D G Q R V P I N S A G G F K L K S A A K E E A E E I E N Q F R H
ACTATTGATGGTCAGCGTGTACCTATCAATTCAGCAGGCGGTTTTAAACTAAAGAGTGCTGCCAAAGAAGAAGCTGAGGAGATAGAAAATCAGTTTCGAC 3101
G T Y V E T T D E S F A A Y Y K K W N E T F W I G K K G P D A D R
ATGGCACTTACGTTGAAACCACCGATGAAAGTTTTGCCGCATATTATAAAAAATGGATGGAAACTTTTTGGATTGGCAAAAAAGGACCAGACGCTGATCG 3201
H Y R D A L K C I E H Y F P H A R M K D I T H D Q Y Q L F I N N F
CCACTATCGTGATGCCCTAAAATGCATTGAACACTATTTTCCGCACGCTCGAATGAAGGACATCACTCATGACCAGTACCAACTATTTATCAATAATTTT 3301
A K S H A K S T V M Q R H N Y I K K C L I E A F E E G I I K R N P A
GCCAAAAGCCATGCTAAGAGCACTGTTATGCAGCGCCATAACTACATCAAGAAATGCTTAATTGAAGCATTTGAGGAAGGCATCATTAAACGAAATCCGG 3401
A R I N L T G N K N R E K K E E V K F N S L D D F K K I H N A A Y
CTGCTAGAATCAATTTGACCGGTAATAAGAACCGGGAGAAAAAAGAAGAAGTTAAATTTATGAGTTTGGATGACTTCAAAAAAATTATGAATGCAGCTTA 3501
R K F D P N S P S T S M I I L M G A T G L R F E E A D G L T W D C
TCGCAAGTTTGACCCAAACTCCCCTTCCACTTCTATGATCATTCTAATGGGGGCAACTGGGCTGCGGTTTGAAGAAGCTGATGGTCTAACTTGGGATTGT 3601
V D F K N S S I T I N K T W D Y R H K H D F G P T K N P Q S N R T I
GTCGACTTTAAGAACAGTAGCATTACCATCAACAAGACCTGGGACTATCGTCATAAACACGACTTTGGCCCCACTAAGAACCCACAGTCAATGCGAACTA 3701
K V D S T T N R V L K A L H A H Y A Q I K L V R H D W N K R K L V
TTAAAGTAGATTCGACAACCATGCGTGTCTTAAAAGCGTTGCACGCCCACTACGCACAAATTAAATTAGTGCGGCATGACTGGAACAAGCGTAAGCTAGT 3801
F S K S D G I P L S N N A I N K M L R S L C V Q N A V V A R N S S
TTTCTCAAAATCAGACGGCATCCCACTATCAAACAACGCAATTAACAAAATGTTGCGAAGCCTGTGCGTTCAAAATGCAGTCGTAGCAAGGAATTCCAGC 3901
G V I Q K W Y T C H A L R H T H A S L L L Y E G R D I S Y V S K R L
GGTGTAATTCAAAAATGGTACACGTGCCACGCCCTCCGTCATACTCACGCATCACTGCTTTTATACGAGGGTCGGGATATTTCTTACGTGTCAAAGCGGT 4001
G H K D I M T T Y N T Y T H V I Q E N S A R E D E A L D P T N S K
TAGGCCATAAGGACATAATGACTACGTACAACACGTATACGCACGTCATCCAGGAAATGAGTGCCCGCGAAGATGAAGCTCTTGATCCAACCATGAGCAA 4101
I F S K Q A *
GATTTTTTCTAAGCAAGCTTAAAATCTCGTGTACGTGTCTGTGTACCTCTTGTGTACGTAAAATAGCAAACGGAAGGTTACAAAAAACATCATGCGCACA 4201
RS-1
RS-2
RS-3
AAAAAAGAACGCCAATCGTTGATATAACAACGATTTAGCGTTCTTCAATCTTACAATAATCAACCGTAAATAACGGTTTGATGACCCCACCCGGACTCGA 4301
IHF
IHF
ACCGGTGTTCAGAGAGCACTGACATATCAGTGTTTAATAGGTGTCACTCAAATATTCGTGTACCTCTCGTGTACCTTTTATATAAAATCAATGTAACGGC 4401
IHF
RS-4
RS-5
CGTGTCGAAAGATACGGCTTTTTCAATATCTAATTGCCTAATTATATCATATAGAACATTTGAATTTACATTCGACAATCAAAACAGGATTTTAAATTAT 4501
Ti1
TTCCAAATAAAAATGAAATGCGAGTCAACTTGCGATTCAGATAGATGTCGGGCCCCCACCCGGCCTATCTGTTTAATTTACCCACGCCTACCCCGCGTGG 4601
Ti2
GTATTTTTTTCGGCAGAAATTTGAGTTCGGCAGCTAACCGCGCTTCTTTAGCCTCTTCAATCGTATCAAAGTAGCCAATACGCGTTCGAACGTGATTAAC 4701
ATAAATCTGGGCCAGGTACTTTGGCCCATTTTTGCGCTGAACCTCGGAAACTCCCGTGACTCCAGTTTTACTATTCTTAGCAACTTTTTTGCGGTTGCTA 4801
Ti3
TCAATCAAGCCCACAGCGACTCCATCGACGAAAAGCTTATCATAGTGCGCCTTCTCCAATGCATTCAACCGCTCGGACGTTTTGAATCCATGACCGCAGC 4901
TTGTGCGTATGCCCTTTACCAGCGGGCTAGCGGGAACTGCAATTTGCTTGCCACAGTCGCACTCGCAAAGCCACAAACTTTTTGGGTGATTATCATCCAC 5001
CGGAATACGCTCAAGAACAGTTAAATGGCCAAACTGACGGCCAGTCAAATCGCCATACTTGTGATTTGTGGGGTGACGATACCTGTCACCACAAGTAGTT 5101
TGACCACCGCTGTTCAGCCGTCCAGTGCTCACTTGTACCTGGTTGCCACAAGTACACTGACACAGCCACATGTCATCACCCGAGTGACTTCGGCCATCCA 5201
TCTTTTTTAGTACCGTCAGATGGCCGAACTGTCTTCCACTCAAGTCTCGCGTCTTACTATTTACTTCTTTTTTCATATCTTATAATTTGGCGCCTAGATT 5301
* L K A G L N
End of Rorf192

Table 1
Putative gene located in the suroundings of the øg1e *attP* site

Gene	Ribosomal binding sequence	Predicted gene product			
		Protein	aa	M_W ($\times 10^{-3}$)	p*I*
Lorf143	AaAAAGtAGGT-gtttacATG	gp*Lorf143*	143	16 333	4.35
Lorf304	AatttGGAGGa-gtaaataATG	gp*Lorf304*	304	33 578	8.75
xis	cGAAAGGAtGT-gtttgtATG	Xis	66	7557	4.55
int	AGtttGGAGGgataaaaacATG	Int	391	45 515	9.70
Consensus	*AGAAAGGAGGT*				

The consensus sequence for *Lactococcus* 16s rRNA is from Ludwig et al. (1985). Capital letters (consensus) indicate the nucleotides identical to those of the 16s RNA. Dashes represent gaps inserted to align nucleotides for maximal homology. ATG, starting codon; aa, number of amino acid residues; M_W and p*I*, molecular weight and isoelectric point, respectively, estimated from DNA sequence.

Rorf148 (Fig. 1), present and previous (Oki et al., 1996a) studies have shown that its deduced product (a 17.0-kDa protein composed of 148 amino acids with a p*I* of 9.80) has an arginine-rich N-terminus, and somewhat resembles the arginine-rich motif found in lambdoid antiterminators (Burd and Dreyfuss, 1994).

3.2. *Characterization of øg1e Int and Xis*

øg1e Int exhibits significant similarity to other putative integrases encoded by the *Lactobacillus* phage øadh (Fremaux et al., 1993) and mv4 (Dupont et al., 1995), and the *Lactococcus* phage øLC3 (Lillehaug and Birkeland, 1993), Tuc2009 (Van de Guchte et al., 1994), and BK5-T (Boyce et al., 1995), as well as the lambdoid phage including λ, ø80, P2, and P22 (Campbell, 1994). For example, overall identity between øg1e and øLC3 is 37%.

The integrase family of lambdoid phages has two highly conserved regions, known as domains 1 and 2 (Sherratt, 1993); the former, located in the central region, contains an Arg residue, whereas the later, situated in the C-terminus, carries a typical Tyr residue, which has been presumed to join to a recessed 3′-phosphate after the initial integrase-mediated phosphodiester cleavage (Sherratt, 1993; Han et al., 1994). Like other integrases, øg1e Int also has two homologous domains 1 (from 204 to 218) and 2 (from 328 to 366), which contain Arg-207 and Tyr-364, respectively (Fig. 4). In Fig. 5, the two domains of øg1e are compared with those of other phages øadh (Fremaux et al., 1993), mv4 (Dupont et al., 1995), øLC3 (Lillehaug and Birkeland, 1993), and λ (Sherratt, 1993).

øg1e Xis shows a resemblance (32% identity and 46% similarity) with a presumed excisionase encoded by the *Staphylococcus* phage ø11 (Ye et al., 1990). Unlike other excisionases of the lambdoid phages λ, P22, and ø80 (Leong et al., 1986) the two Xis proteins of øg1e and ø11 are significantly acidic (Table 1), suggesting that øg1e Xis functions via a mechanism different from the lambdoid excisionases, as in ø11 (Campbell, 1994; Ye et al., 1990).

3.3. *Properties of the øg1e attP region*

The regions preceding and following *xis-int-attP* are rich in inverted sequence and secondary structure. An intergenic region between *Lorf304* and *xis* contains three successive secondary structures (Fig. 4), which may function in control of the *xis* and/or *int* expression. In coliphage P2 (Yu et al., 1994), three secondary structures located just upstream of *int* has been predicted to be involved in control of the *int* expression. In øg1e *xis*, there exists an imperfect secondary structure preceded by mRNA promoter-like sequences (see Fig. 4), which may function in the *int* expression during the integrative pathway, like the λ *int* system (Campbell, 1994).

DNA regions flanking øg1e *attP* have also several inverted and/or direct repeats (Fig. 4). Three possible secondary structures (*Ti*) located downstream of *attP* end in a run of T residues, and somewhat resemble a ρ-independent mRNA terminator of lambda *int* (Guarneros et al., 1982). On the other hand, this DNA region of øg1e contains five homologous repeated sequences (*RS*), each of which is composed of 11 bases with a consensus of 5′-TNGTGTACNTN-3′: three of them (*RS1-RS2-RS3*) are located upstream of *attP*, and another two (*RS4-RS5*) are situated downstream of *attP*. In addition, *attP* is accompanied by three possible IHF-binding sequences: two of them are localized between *RS-3* and *attP*, and the other one is between *attP* and *RS-4*. These IHF-binding-like sequences of

Fig. 4. DNA sequence of the øg1e *xis-int* region. A 2800-bp DNA of the 5204-bp *Sac*I–II fragment (see Fig. 1) was shown together with deduced amino acid sequences of *Lorf304-xis-int-Rorf192*. The 24-bp core sequence (*attP*) was boxed (see text). Asterisks indicate stop codons. Bold italic lower letters indicate putative ribosomal binding sites (RB). Arrows show inverted repeats. *Ti*, possible ρ-independent mRNA terminator. Dotted double lines represent repeated sequences (RS). IHF, potential IHF-binding site.

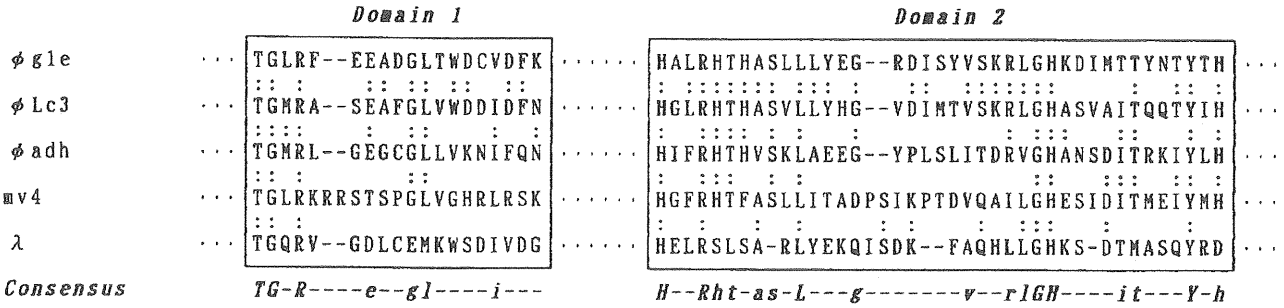

Fig. 5. Comparison of øgle Int with other integrases. The two conserved regions (domains 1 and 2) among the phage-encoded integrases were compared: øgle (this study), the *Lactococcus* phage øLC3 (Lillehaug and Birkeland, 1993), the *Lactobacillus* phage øadh (Fremaux et al., 1993) and mv4 (Dupont et al., 1995), and the *E. coli* phage λ (Sanger et al., 1982). Domains 1 and 2 (boxed) were from Sherratt (1993). Dashes indicate positions with conservative substitutions. Consensus indicates the conserved aa residues; capital letters are conserved through the intergases.

øgle are apparently similar to the *E. coli* IHF-binding consensus of 5′-(C/T)AANNNNTTGAT(A/T)-3′ (Landy, 1989).

3.4. *Expression of the øgle int* product in *Escherichia coli*

As shown in Fig. 1, p18intScL (see above) carries *int* under *plac*, but this plasmid did not overproduce Int in *E. coli* XL1-blue cells, by treatment with IPTG (data not shown). Therefore, a DNA region of 1585 bp spanning from 2901 to 4485 (Fig. 4) was cloned into a *Sma*I site of pUC18, resulting in two plasmids, p18intL (under *plac* control) and p18intR (free from *plac*) (Fig. 1): the cloned øgle DNA region contains a possible ribosomal binding site (5′-GGAGG-3′), *int*, *attP*, and one putative mRNA terminator *Til* (Fig. 4). Unlike p18intScL, *E. coli* XL1-blue cells carrying p18intL cannot grow stably at 37°C even without induction, but multiply normally at 25°C.

Total content of proteins extracted from XL1-Blue/p18intL was analyzed by SDS PAGE (Section 2). Upon induction by IPTG (1 mM) at 37°C, p18intL directed synthesis of a protein increasing steadily; its apparent molecular mass is 42.0 kDa (Fig. 6). This protein was not detected when IPTG was omitted or the cells harboring p18intR or pUC18 were used. The overproduced protein is probably Int, although the mass on an SDS gel was slightly lower than that (45.5 kDa) predicted from DNA sequence (Table 1). By gel mobility-shift assay, a cell-free extract from XL1-Blue/p18intL resulted in a lower mobility of a DNA fragment containing the *attP* region specifically (e.g., a 90-bp *Hin*dIII/*Fsp*I fragment from nucleotide positions 4117 to 4196, see Fig. 4) (unpublished results).

The results in this study suggested that the two øgle putative proteins, Int and/or Xis, are involved in the phage movement into or out of the host chromosome. Int probably acts as a site-specific recombinase in both excision and integration using two specific attachment

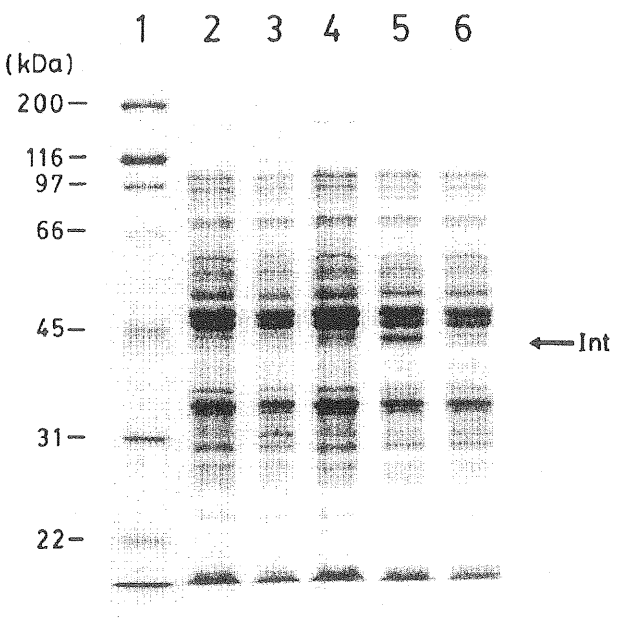

Fig. 6. Expression of øgle Int in *E. coli*. *E. coli* XL1-blue carrying p18intL, p18intR or pUC18, was grown at 25°C in LB medium containing ampicillin (60 μg/ml). When the A_{660} of the culture had reached 0.5, the growth temperature was shifted to 37°C with concomitant addition of IPTG (final concentration 1 mM). At intervals, a portion of the culture was removed, disrupted, and subjected to 10% SDS PAGE (Section 2). Lane 1, markers; lane 2, no plasmid (after 3 h); lane 3, pUC18 (3 h); lane 4, p18intL (1 h); lane 5, p18intL (3 h); lane 6, p18intR (3 h). An arrow indicates the putative Int overproduced.

sites, *attP* and *attB*, locating on the phage genome and on the host chromosome, respectively. Like the ø11 excisionase (Ye et al., 1990), øgle Xis, an acidic protein, may participate in the excisive pathway, through a cooperation with Int. As in λ (Landy, 1989), the øgle integrative and excisional pathways are probably under a complicated control. Further studies on structure, expression, and function of øgle *int* and *xis* are now in progress.

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