Transposition burst of the ISH27 insertion element family in *Halobacterium halobium*

Felicitas Pfeifer* and Ulrike Blaseio
Max-Planck-Institut für Biochemie, D-8033 Martinsried, FRG

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ABSTRACT

Investigation of the plasmid pHH4 in single colonies of *Halobacterium halobium* PHH4 indicated transposition of insertion elements in 20% of the colonies. Seven ISH27 insertions were observed as well as one ISH23 insertion. The various copies of ISH27 were compared to the two ISH27 elements already present in pHH4, and to the ISH27 element that was identified in the bacterio-opsin (*bop*) gene of a Bop mutant. These ten copies of ISH27 constitute three types on the basis of DNA sequence identity: ISH27-1 (1398 bp), ISH27-2, and ISH27-3 (1389 bp each). The DNA sequence comparison between the three types indicates a region of 1200 bp where the identity between ISH27-1 and ISH27-2 or ISH27-3 is 82–83%. ISH27-2 and ISH27-3 are 95% identical in this region. The remaining region exhibits a lower DNA similarity (64–74% identity) between the different copies. An open reading frame of 1167 nucleotides spans the more conserved region, and a corresponding transcript could be detected in *H. halobium* PHH4, but not in *H. halobium* wild-type. ISH27-1 is 91% identical to members of the insertion sequence-like elements ISH51 of *Haloferax volcanii*, whereas the other two ISH27 element types are 82–83% identical to ISH51. The transposition 'burst' of ISH27 was only seen after storage of the cells for more than two years at 4°C. Upon continuous cultivation at 37°C no transposition event could be observed, suggesting that stress factor(s) might have caused the high transposition rate.

INTRODUCTION

The halophilic archaebacterium *Halobacterium halobium* contains a number of different transposable elements, seven of which were identified in the bacterio-opsin (*bop*) gene region of various Bop mutants (1–5). Some of these ISH-elements (ISH1, ISH2, ISH23/ISH50, and ISH26) have been characterized by DNA sequence determination (1,2,6,7). *H. halobium* harbors copies of these elements mainly in a DNA fraction (FII-DNA) that is by 10% lower in guanosine and cytosine (G+C) content than the majority of the genomic DNA (FI-DNA, 68% G+C). The FII-DNA (58% G+C) encompasses 30% of the total *H. halobium* DNA and consists of plasmid DNA as well as some chromosomal DNA regions (8,9). *H. halobium* wild-type contains a heterogeneous population of covalently closed circular DNA (cccDNA) varying in size from 150–200 kbp, with a major plasmid species of 150 kbp (pHH1) that is present at 6–8 copies per chromosome (10). In *H. halobium* wild-type this plasmid harbors copies of all ISH-elements except ISH1 and ISH1.8 (11,12). Analysis of deletion events in plasmid pHH1 of *H. halobium* wild-type and the smaller derivative pHH4 indicated that the deletions result from abortive transpositions (13,14). Among the 40 single colonies analyzed for the presence of smaller plasmids, we detected insertions into the pHH4 plasmid in eight cases, and once an insert in the smaller derivative, pHH6 (13,14; and this report).

In this paper we describe the analysis of these insertions that with one exception belong to the ISH27 insertion element family. The DNA sequences of ten ISH27 elements were determined and compared to each other and to members of the related, degenerate family of insertion sequence-like elements ISH51 of *Hf. volcanii* (15). The ISH27 elements could be assigned to three types (ISH27-1, ISH27-2, ISH27-3) on the basis of their DNA sequence similarity.

MATERIAL AND METHODS

Material

Restriction endonucleases, T4 DNA ligase, and T4 DNA polymerase were purchased from Boehringer Mannheim. α-^{32}P dATP, α-^{35}S dATP were purchased from Amersham. Nylon membranes used for Southern and Northern transfers were obtained from Pall. T7 polymerase and the Sequenase™ sequencing kits were from U.S. Biochemicals. The 'Geneclean' kit used for the isolation of DNA fragments from agarose gels was obtained from BIO. All other chemicals were obtained from Sigma or Merck.

Bacterial strains and plasmids

H. halobium wild-type and *H. halobium* PHH4 were described previously (13). The proper designation for H. halobium according to Bergey's manual is now Hb. salinarium. The plasmid content of the H. halobium strains, however, differs from the plasmids found in Hb. salinarium type strains. *Hf. volcanii* WFD11, and the pHV2-derived plasmid pWL101 containing ISH51-3 (16) were provided by W.F. Doolittle.

* To whom correspondence should be addressed

DNA isolation and DNA sequence determination

Construction of recombinant DNA, transformation into *Escherichia coli* DH5a, and DNA isolations were carried out as described in Sambrook et al (17). The isolation of halobacterial DNA (total DNA and plasmid DNA) was done as described (13). DNA sequence determinations were performed using the Sequenase™ system as recommended by the manufacturer. The following synthetic oligonucleotide primers complementary to internal ISH27 sequences were used to determine internal ISH27 or ISH51-3 sequences: (5'-3') GCGGGGACGGCCCGACG, GCGGGACGGCCCGACGC, CTCACCACGACG-TACAG, AGAAACTCTGCGAGGAC, CG(GT)AGATG(GA)TAGA-GAA, AGAAGCTGGTCCTCGTG, CAACGGGCAGCGT-GGAC, CCTCTGG(TA)(GA)-GTGG(CT)CGT, CTGTACGT-CGTGGTGAG, GTCTACATCGACTGTAC, TGTGAAGAA-CAAACGCT, TATCACTCACAAGCGAA, GGACCTCGGT-CTCA-ACGCT, TGCTGAAGGTGTGTCTA. For computer-aided editing and alignment of DNA sequences the programs of Devereux et al (18) were used. The nucleotide sequence data reported will appear in the EMBL nucleotide sequence data base under the accession number X54432 (ISH27-1), X54433 (ISH27-2), and X54434 (ISH27-3).

Southern and Northern analysis

DNA probes were labelled by the procedure described by Feinberg and Vogelstein (19). Transfer on to nylon membranes and hybridizations were done as described in Pfeifer et al (13). RNA isolation and electrophoretic separation (on glyoxal gels) was done as described in Sambrook et al (17) and Englert et al (20).

RESULTS AND DISCUSSION

Analysis of plasmids altered by insertion elements

The analysis of 40 single colonies of *H. halobium* PHH4 indicated that seven pHH4 plasmids as well as the smaller deletion variant pHH6 incurred a DNA insertion (13). The altered plasmids were isolated, and comparative restriction mapping indicated size increases of 1.4 kbp (seven times) or 1 kbp (once) in various subfragments (Fig. 1). Fragments containing the additional DNA were hybridized to the various ISH-elements isolated from the *bop* gene region of Bop mutants (3−5) in Southern analyses. The seven 1.4 kbp inserts each hybridized with ISH27 isolated from Bop mutant O5 (5), whereas the 1 kbp insert hybridized with ISH23 found in Bop mutant IV-10 (4) (data not shown, see Fig. 1).

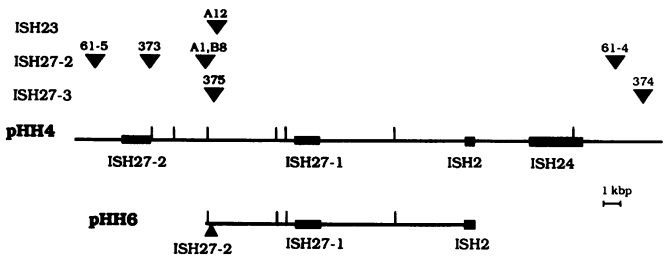

Figure 1: *Cla* I restriction map of plasmids pHH4 and pHH6. The integration sites of ISH23 and various ISH27 insertion elements in the variants are marked by triangles. The designation of the respective variant harboring this altered plasmid is indicated above the triangle. ISH-elements present in the original pHH4 and pHH6 are marked by black boxes.

Target DNA and DNA sequence determination of the various insertion elements

Preliminary DNA sequence determination of the ISH23 element integrated into the pHH4 plasmid of variant A12 indicated an almost identical DNA sequence to the ISH23/ISH50 element (4,6). The 9 bp target DNA duplication of the ISH23 element in pHH4 is shown in Table 1. The target DNA differs in sequence and length from the target site found for ISH23 in Bop mutant IV-10 (4) or the related ISH50 element located in the pHH1-type plasmid of strain R1 (6) (Table 1).

The complete DNA sequence was determined for the ISH27 element of Bop mutant O5, the two copies of ISH27 present in pHH4, as well as for each of the seven ISH27 elements found in pHH4 variants and pHH6 (see Fig. 1). Each ISH27 element contained the same 16 bp inverted repeat sequence (with 1 bp mismatch) at the terminus, and all except the ISH27-2 element present in pHH4 showed a 5 bp duplication of target DNA (Table 1). Except for variant A1, where the ISH27-2 target DNA duplication was 5'AGTCC 3', the motif 5'ANNNT 3' was always found (Table 1). The constant length of 5 bp target DNA is unusual for halobacterial insertion elements. Most often target DNA of various lengths are duplicated with ISH2 (10 bp, 11 bp, 12 bp or 20 bp) (2,4,14), ISH23/ISH50 (8 bp or 9 bp) (4,6, and this report) and ISH26 (11 bp or 9 bp) (7, and unpublished results). Due to a deletion event in the pHH4 precursor plasmid, the ISH27-2 element in pHH4 is flanked by different 5 bp DNA sequences at both termini (14). The sequences on both sides, however, exhibited the motif 5'ANNNT 3', which led to the conclusion that the deletion resulted from an abortive ISH27-2 transposition (14).

Five insertions (ISH27 in variants A1, B8, 375, and pHH6, and an ISH23-element in variant A12) and three deletion events occurred in a 400 bp 'hot-spot' region located 4.6 kbp apart from the ISH27-1 element of pHH4 (Fig. 2). The adenosine plus thymidine content of this region (53% A+T) is higher than the average A+T content of halobacterial DNA (32% A+T for FI-DNA, and 42% for FII-DNA). The deletions led to the smaller pHH4 derivatives pHH6 (17 kbp), pHH8 (6.3 kbp) (13,14), or the pHH1 variant pHH23 (65 kbp). The deletion event leading to pHH23 was also due to an abortive transposition of ISH27; the sequence 5'AGTCT 3' was fused to ISH27 (see Fig. 2B).

Table 1: DNA sequences of the target DNAs of ISH-elements at various integration sites. The target DNA of ISH23 in Bop mutant IV-10 and ISH50 were taken from reference 4 and 6, respectively.

ISH-element	integration site in:	target site
ISH23	pHH4, variant A12	5'TCTTGGTTA 3'
ISH23	Bop mutant IV-10	5'TACACACAT 3'
ISH50	R1 plasmid	5'TTGTGGAT 3'
ISH27-1	Bop mutant O5	5'ATCCT 3'
ISH27-1	pHH4	5'AGGTT 3'
ISH27-2	pHH4, variant 373	5'AGAGT 3'
ISH27-2	pHH4, variant 61-4	5'AACCT 3'
ISH27-2	pHH4, variant 61-5	5'AGTAT 3'
ISH27-2	pHH4, variant A1	5'AGTCC 3'
ISH27-2	pHH4, variant B8	5'AAGCT 3'
ISH27-2	pHH6	5'ATTCT 3'
ISH27-3	pHH4, variant 374	5'AGACT 3'
ISH27-3	pHH4, variant 375	5'ATTCT 3'

Table 1: DNA sequences of the target DNAs of ISH-elements at various integration sites. The target DNA of ISH23 in Bop mutant IV-10 and ISH50 were taken from reference 4 and 6, respectively.

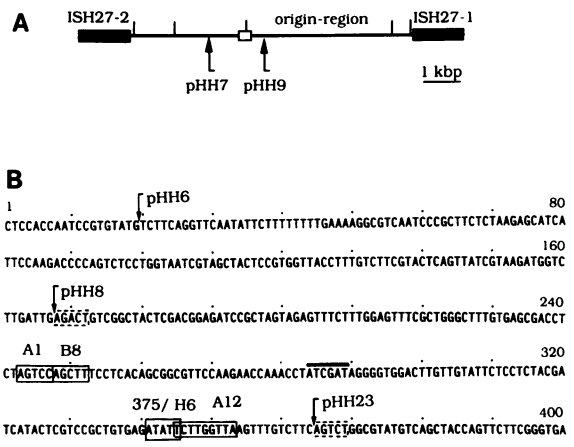

Figure 2 A,B: *Cla* I restriction map of the region located between ISH27-2 and ISH27-1 in pHH4 (A), and the 400 bp 'hot-spot' region (B). (A) The two ISH27 elements are indicated by black boxes, while the 400 bp DNA sequence shown in (B) is represented by a white box. Arrows mark the sites of the deletions leading to pHH7 and pHH9 (14). (B) DNA sequence of the 400 bp region that incurred five insertions and three deletion events. The 4.6-kbp-region (origin region) between ISH27-1 in pHH4 and the 'hot-spot' region contains the functions for autonomous plasmid replication (21). The target DNA of the ISH-elements integrated in pHH4 of the variants A1, A12, B8, 375 and pHH6 (designated H6) are boxed, and the sites of the deletion events leading to the plasmid variants pHH6, pHH8, and pHH23 are indicated by arrows. The *Cla* I site present in this region is marked by a black line.

Two other deletion events leading to plasmids pHH7 (16 kbp) or pHH9 (5.7 kbp) occurred at sites less than 1 kbp apart from this region (Fig. 2A). No mutation event was observed in the 4.6 kbp region separating ISH27-1 and the region involved in the formation of pHH9; this DNA sequence contains all functions necessary for autonomous plasmid replication as demonstrated by transformation experiments (21).

DNA sequence comparison of the ISH27 elements

The ten copies of ISH27 elements could be assigned to three types [ISH27-1 (1398 bp), ISH27-2, and ISH27-3 (1389 bp each)] that showed similar but not identical DNA sequences (Fig. 3). Elements of the same type exhibited identical DNA sequences. For the first 1200 bp of the ISH27 sequence shown in Fig. 3, these various types were identical by 83% or 82% (ISH27-2 and ISH27-3, respectively with ISH27-1) or more closely related (95% DNA sequence identity between ISH27-2 and ISH27-3). A higher degree of diversity was found for the last 200 bp of the elements; the DNA sequence identity in this region was between 64 and 74% (Fig. 4). An open reading frame of 1167 nucleotides (ORF1167) was found within the more conserved region of each ISH27 element, with a GTG start codon and a TAG or a TAA stop codon (see Fig. 3). The deduced amino acid sequences are shown in Fig. 5. The protein encoded by ISH27-2 is 98% similar to the protein of the ISH27-3 element

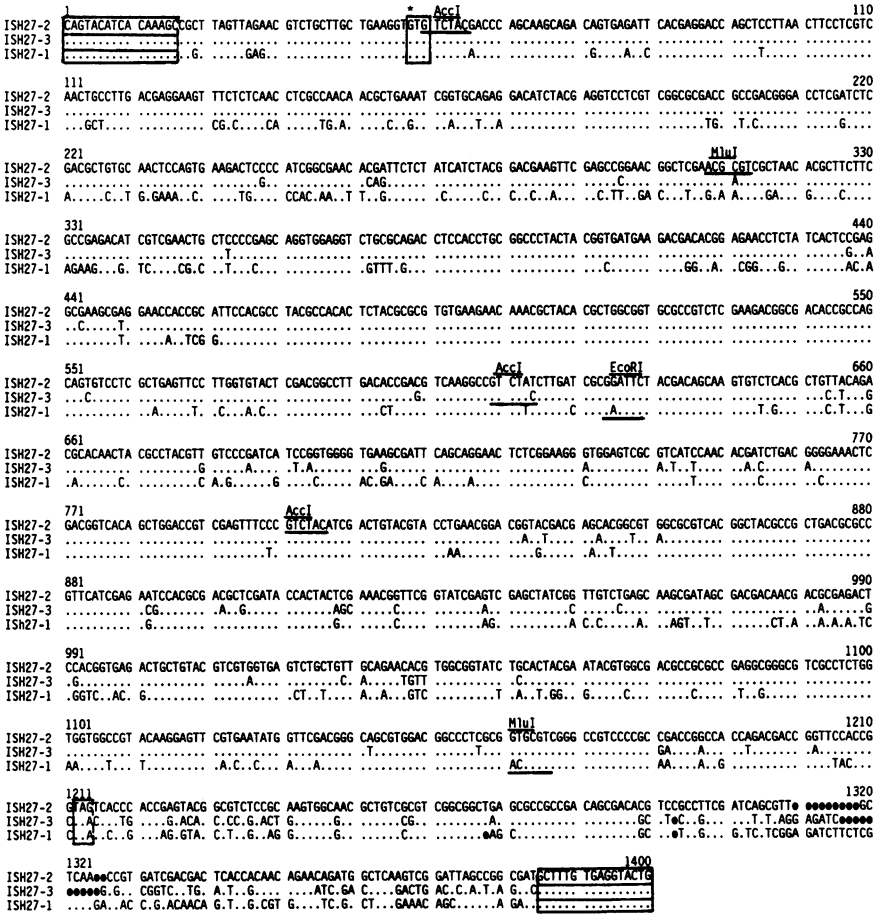

Figure 3: DNA sequence alignment of the three ISH27 element types. The inverted repeat sequences at the termini are boxed. *Acc* I, *Mlu* I, and *Eco*RI restriction sites are marked as well as the GTG start codons of ORF1167, and the stop codons. Dots represent bases identical to the sequence given on top, whereas filled in '○' denote gaps introduced to obtain an optimal alignment.

type, and 90% similar to the product of ISH27-1 (or ISH51-3, see below).

Comparison of ISH27 with the family of insertion sequence-like elements ISH51 of *Haloferax volcanii*

The inverted repeat sequence of ISH27 is identical to the inverted repeat determined for members of the degenerate family of the insertion sequence-like elements ISH51 of *Hf. volcanii* (15). The complete DNA sequences of two copies (ISH51-1 and ISH51-2) have been determined; however, the ability of these elements to transpose has not been demonstrated (15). The alignment of DNA sequences of various ISH27 elements with ISH51-1 and ISH51-2 indicated a high degree of DNA sequence identity (90%) between ISH27-1 and the two members of the ISH51 family, whereas the similarity between ISH27-2 or ISH27-3 and the ISH51 elements was 82–84% (Fig. 4). Thus, ISH27-1 is more closely related to members of the ISH51 family (90%) of *Hf. volcanii* than to the other ISH27 element types (82–83%) found in the same *H. halobium* cell. Furthermore, the alignment of the various ISH27 elements and the two ISH51 elements indicated about 30 insertions or deletions of one or more base pairs in the DNA sequence of the two ISH51 elements (data not shown). Thirty of these alterations occurred at different positions in either ISH51-1 or ISH51-2; these mutations disrupt the ORF1167 found in ISH27 and may be the reason for a lack of transposition functions in ISH51-1 and ISH51-2 (15).

Recently, transposition of an ISH51 element (ISH51-3) was found into plasmid pHV2 of *Hf. volcanii* (16). DNA sequence determination of the region in ISH51-3 corresponding to ORF1167 in ISH27 indicated none of the single base pair insertions or deletions found in the sequence of the other two ISH51-elements (data not presented), except for a deletion of 21 bp found at position 420 of ORF1167. The deduced protein sequence (7 amino acids shorter than the respective ISH27 proteins) is shown in Fig. 5 in comparison to the protein sequences deduced from the DNA sequences of ISH27 types. This result suggests that the product of ORF1167 is necessary for the transposition process. The ISH51-3 sequence (like ISH51-1 and ISH51-2) was more related to ISH27-1 (91% identity) than to the other two ISH27 types (83%) (Fig. 4).

Analysis of ISH27 transcripts

To analyze the expression of ORF1167, total RNA of *H. halobium* wild-type, *H. halobium* PHH4, and *Hf. volcanii* WFD11 was isolated at various time points throughout the growth cycle, and probed with strand-specific probes of ISH27-1 in Northern analysis (Fig. 6). RNA corresponding to ORF1167 could be detected in total RNA of *H. halobium* PHH4; we found hybridization signals corresponding to transcripts of 1200 and 650 nucleotides, with a maximal amount in late logarithmic phase of growth (Fig. 6, lanes 1–4). The 650 nucleotide transcript signal appeared to be twice as strong as the signal of larger transcript. No transcripts were detected in total RNA from *H. halobium* wild-type or *Hf. volcanii* (Fig. 6, lanes 5–11). In contrast, probes derived from the opposite DNA strand did not reveal any detectable hybridization signal (data not shown). Thus, there was a high expression of ORF1167 in *H. halobium* PHH4, whereas the other strains and species tested did not contain a detectable amount of ISH27/ISH51 RNA.

No transposition events occur in a continuously grown culture

The analysis of pHH4 in 40 single colonies indicated that about 20% (8 of 40) of the plasmids were altered by additional

	ISH27-1	ISH27-2	ISH27-3	ISH51-1	ISH51-2	ISH51-3
ISH27-1		83	82	90	90	91
ISH27-2	64		95	84	84	82
ISH27-3	74	68		82	82	83
ISH51-1	80	68	68		92	92
ISH51-2	80	68	68	86		92

Figure 4: Percent DNA sequence identity of ISH27 and ISH51 insertion elements. The dark shaded half indicates the identity values of the first 1200 nucleotides (conserved region), whereas the other half gives the values for the last 200 bp (variable region).

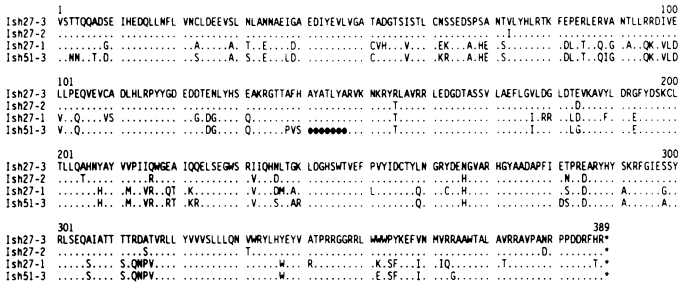

Figure 5: Alignment of the deduced amino acid sequence of ORF1167 in the three ISH27 elements and ISH51-3. Dots represent amino acids identical to the sequence given on top, whereas missing amino acids are marked by '○'.

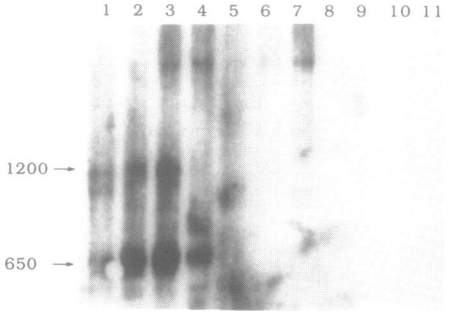

Figure 6: Northern analysis using a strand-specific probe (complementary to ORF1167 RNA) against total RNA of *H. halobium* PHH4 (lanes 1–4), *H. halobium* wild-type (lanes 5–7), and *Hf. volcanii* WFD11 (lanes 8–11). Samples were taken at various time points during the growth; the OD_{600} values of the cultures were: lane 1, early log. phase, OD=0.41; 2, late log. phase OD=0.88; 3, early stationary phase, OD=1.85; 4, stationary phase, OD=2.1; 5, OD=0.88; 6, OD=1.53; 7, OD=1.88; 8, OD=0.39; 9, OD=1.33; 10, OD=1.53, and 11, OD=1.60. The transcript sizes detected for *H. halobium* PHH4 are given in nucleotides.

insertions. This extremely high frequency of transposition was found in a culture that had been stored at 4°C for about two years, conditions that might have imposed stress on the cells. To test whether these insertions were due to a stress-induced transposition 'burst' or whether *H. halobium* PHH4 is a deregulated ISH27 transposon mutant, we studied the transposition frequency in the strain during continuous growth at 37°C for 3 months. Every month 12 single colonies were isolated and investigated for plasmid alterations. None of the 36 colonies analyzed indicated size alterations in subfragments of pHH4 (data not shown).

These experiments demonstrate that the extremely high frequency of ISH27 transpositions observed during the initial

analysis of the strain were due to a 'burst' of transpositions rather than a generally high transposition rate of ISH27 elements in *H. halobium* PHH4. The possibility of a selection against halobacteria with insertions in their plasmids in the latter experiment appears to be unlikely since the possession of pHH4 is not vital to the cell. Furthermore, bacterio-opsin mutants with insertion elements in the chromosomal bop gene usually also contain additional insertions in the plasmid DNA (3,4). Nothing is known about the factor(s) that might have induced this wave of ISH27 transpositions in the initial culture of H. halobium PHH4. Further investigations are necessary to determine the cause of these differences in transposition activity in more detail.

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