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Sequence analysis of the insertion element ISH1.8 and of associated structural changes in the genome of phage ΦH of the archaebacterium *Halobacterium halobium*

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We have sequenced the insertion element ISH1.8 which can be present in one or two copies in the genome of phage ΦH of *Halobacterium halobium*. ISH1.8 is 1895 bp long, has no inverted repeat at its ends, and one only of the two copies is flanked by two 5-bp duplications. An 8-bp sequence composed of 4 bp from each end of ISH1.8 is present in both sites lacking the element. This 8-bp sequence could either be a specific insertion sequence or a part of the element that is left behind upon deletion. The plasmid pΦHL, consisting of the invertible L segment of the phage genome which is, in ΦH2 and ΦH5, flanked by two copies of ISH1.8, contains 112 bp of ISH1.8 and is released from the phage genome by recombination within a direct repeat of 9 bp. This 9-bp sequence (TCCCGCCCT) exists as an inverted repeat in ISH1.8 and therefore as two distinct repeats in phage genomes containing two copies of ISH1.8 in inverted orientation.

Key words: archaebacteria/insertion element/phage ΦH variants/genome organization/*Halobacterium halobium*

Introduction

Several viruses that can serve as models for genome organization and gene expression in archaebacteria have been described for *Halobacterium* (Wais *et al.*, 1975; Torsvik and Dundas, 1980; Schnabel *et al.*, 1982a; Pauling, 1982) and for *Thermoproteus tenax* (Janekovic *et al.*, 1983) and a virus-like particle has been identified in *Sulfolobus acidocaldarius* (Martin *et al.*, 1984). The temperate phage ΦH of *Halobacterium halobium* is the only one studied in detail on the molecular level. Its 59-kb genome is terminally redundant and partially circularly permuted due to a headful packaging mechanism (Schnabel *et al.*, 1982a). In lysogens, the prophage genome is a covalently closed circle of 57 kb (Schnabel and Zillig, 1984).

Phage ΦH is a mixture of variants differing in the structure of their genomes (Schnabel *et al.*, 1982b). Their analysis is one of several ways in which the extremely high variability of genome structure in *H. halobium* has been studied. Other approaches are the analysis of the plasmid pHH1 (Pfeifer *et al.* 1981), the characterization of bacteriorhodopsin-mutants which has led to the isolation of several transposable elements (Simsek *et al.*, 1982; DasSarma *et al.*, 1983; Pfeifer *et al.*, 1984), and the analysis of repetitive DNA in *H. halobium* (Sapienza *et al.*, 1982).

Of the described phage variants, the predominant variant ΦH1 contains one copy of the insertion sequence ISH1.8. An additional, inverted copy of ISH1.8 is found in the genomes of ΦH2 and ΦH5. We have now isolated two phage variants derived from ΦH2 which contain only this latter copy of ISH1.8 and lack the copy present in ΦH1.

Two recombination events occur with high frequency in phage genomes containing two inverted copies of ISH1.8. One is the inversion of the enclosed L segment (Schnabel *et al.*, 1982b), the other the circularization of the L segment to the 12-kb plasmid pΦHL, which causes a partial immunity of cells against phage infection (Schnabel, 1984). We report here the complete sequence of ISH1.8 and the analysis of sequences involved in structural changes in the phage genome caused by ISH1.8.

Results

Isolation of phage variants lacking ISH1.8

As described earlier (Schnabel *et al.*, 1982b), two single plaques obtained by plating progeny of the phage variant ΦH2 gave rise to mixtures of ~90% of ΦH2 and ~10% each of ΦH7 or ΦH8, indicating that ΦH7 and ΦH8 were generated from ΦH2 during growth of the plaque. ΦH7 and ΦH8 were purified from single plaques of these mixtures. Restriction patterns of their DNAs in comparison with ΦH2 DNA show that, in ΦH8 DNA, one new fragment appears in each restriction pattern, replacing a large ΦH2-fragment (Table I, restriction patterns not shown). The affected fragments are *Pst*I-6, *Cla*I-10 and *Bgl*II-1, all covering a region around 75% map units which contains one copy of ISH1.8 (for restriction fragment numbering and map see Schnabel *et al.*, 1982b). The size decrease of ~1.9 kb (Table I) already indicated that ISH1.8 was deleted in ΦH8 DNA. In ΦH7 DNA, the following fragments are missing: *Pst*I-6 and -10, *Cal*I-1, -8 and -10, and *Bgl*II-1. Their replacement by a single new fragment shows that one *Pst*I and two *Cla*I recognition sites were lost. The fragment sizes in Table I show that a piece of DNA of 4 kb was deleted in ΦH7. In conclusion, ΦH7 and ΦH8 DNA have deletions of ~1.8 kb and 4 kb, respectively, both at 75% map units, where a copy of ISH1.8 is present in all phage variants so far analyzed (Figure 1). No phenotype difference between ΦH7 or ΦH8 and other phage variants were observed.

Table I. Sizes of restriction fragments changed in the phage variants ΦH7 and ΦH8

	ΦH2		ΦH8		ΦH7	
	Fragment number[a]	Size[b]	Size	Size decrease	Size	Size decrease
*Pst*I	6	4.0	2.1	1.9		
	10	1.4	1.4	–	1.5	3.9
*Cla*I	1	11	11	–		
	8	3.3	3.3	–	1.3	3.8
	10	2.5	0.6	1.9		
*Bgl*II	1	8.8	6.9	1.9	4.9	3.9

[a]Fragment numbers according to decreasing size in ΦH1 DNA.
[b]All sizes in kb.

Sequence analysis of ISH1.8

A 2.5-kb *Cla*I-fragment of ΦH1 DNA (*Cla*I-10 at 75% map units) containing one copy of ISH1.8 (position B in Figure 1) was sequenced by the chain termination method (for details see Materials and methods). In order to define the ends of ISH1.8, this sequence was compared with the sequences of the flanks of ISH1.8 at position A (Figure 1). A 157-bp *Sal*I/*Hpa*I fragment containing the left end and a 302-bp piece from an *Alu*I to a *Sal*I restriction site containing the right end of the ISH1.8 from position A in ΦH2 DNA (at 50% map units) were sequenced. Figure 2 shows the restriction sites and the positions of the sequenced fragments. Assuming that all nucleotides identical in positions A and B belong to ISH1.8, the size of the element is 1895 bp (Figure 3). The comparison confirms that the two copies of ISH1.8 in positions A and B are in inverted orientation. Features of ISH1.8 are the absence of an inverted repeat at its ends and the presence of a 5-bp duplication in the sequence adjacent to the element at position B but not at position A. The 5-bp duplication in site B is flanked by another 5-bp duplication.

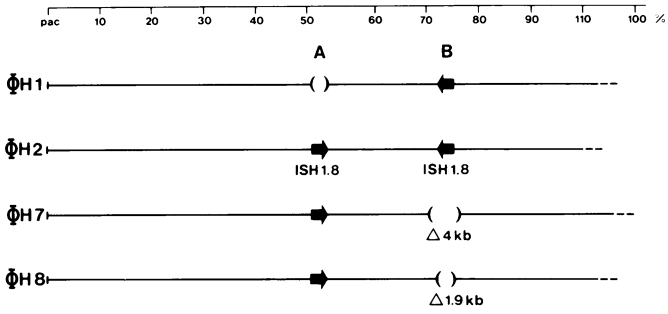

Fig. 1. Genome structure of the variants ΦH7 and ΦH8 in comparison with ΦH2 and ΦH1 DNA. Arrows represent ISH1.8; where they point towards each other, the elements are in inverted orientation. Parentheses indicate absence of DNA, values below are the sizes of the missing DNA segments. In the genomes of the phage variants ΦH7 and ΦH8, both of which arose from ΦH2, ISH1.8 was deleted from site B, in ΦH7 DNA together with ~2 kb of flanking DNA. ΦH1, the predominant variant of the original mixture, lacks ISH1.8 at site A. The ends of these four DNAs differ because the genome length of 59 kb is determined by packaging of headfuls. (Top line: map units in % referring to ΦH1 DNA.).

The element contains several open reading frames in both strands. The longest, beginning at nucleotide 1165 and ending at 1836, would give rise to a protein of 224 amino acids. In addition, there is one open reading frame reading out of the element and one reading into the element (Figure 3). The sequence of ISH1.8 contains 15 direct and 14 inverted repeats of at least 9 bp, spaced by sequences of various lengths.

Sequence analysis of variant genomes lacking ISH1.8

A 310-bp *Sal*I-fragment of ΦH1 DNA containing site A without ISH1.8 and a 214-bp *Xho*I fragment of ΦH8 DNA containing site B without ISH1.8 were sequenced. Comparison with the sequence of ISH1.8 shows that, in both cases, eight nucleotide pairs, four from each end of the element, are found in the empty sites (Figure 4). The 8-bp sequence CGGAATAG displays no repeated or symmetric structure.

Sequence analysis of pΦHL

Phage variants which contain two copies of ISH1.8 at positions A and B are able to generate a plasmid pΦHL which comprises the 12-kb segment between the two ISH1.8 elements (Schnabel, 1984). To elucidate the mechanism of plasmid formation, we sequenced a 382-bp *Xho*I/*Sal*I frag-

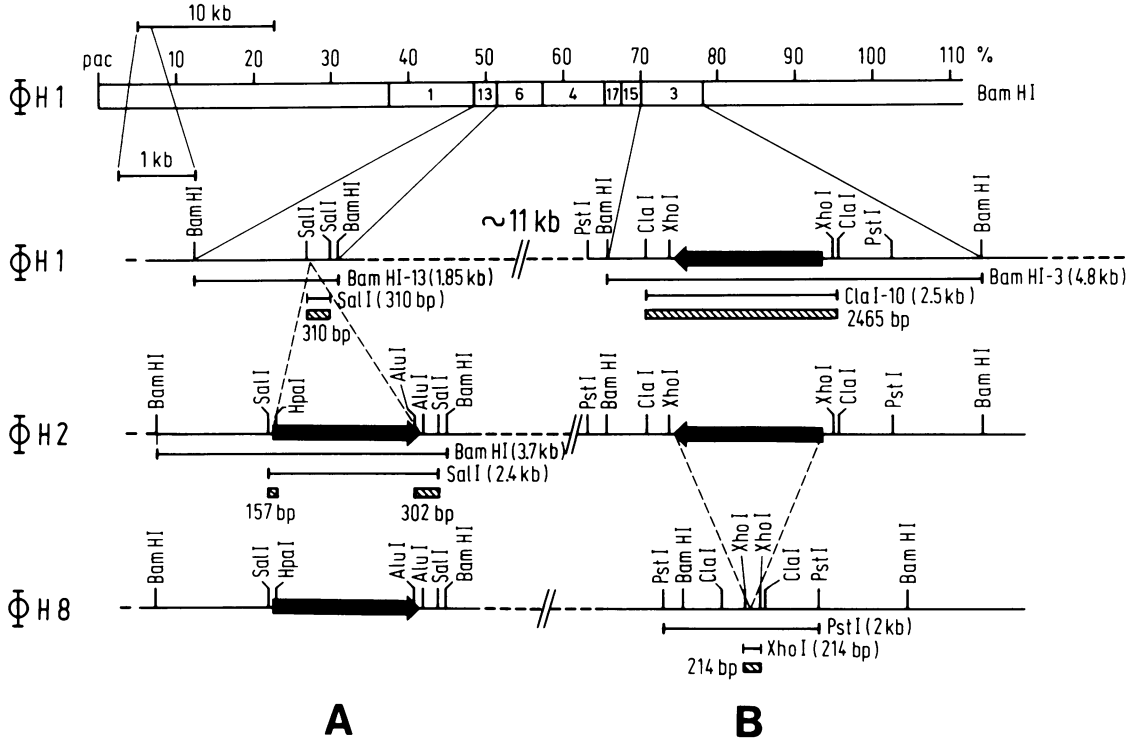

Fig. 2. Map of fragments and cleavage sites used for cloning and sequencing. The genome of ΦH1 is shown on top with part of the *Bam*HI map. The two sites A and B in each of the variants ΦH1, ΦH2 and ΦH8 are represented in larger scale. Dashed lines represent integration or deletion of ISH1.8. Only those restriction sites that were used for cloning and/or sequencing are included, most of the enzymes have additional cleavage sites in these DNAs. The size and number (in ΦH1 DNA) of the fragments that were cloned or isolated are indicated below the maps, regions that were sequenced are shown as hatched boxes.

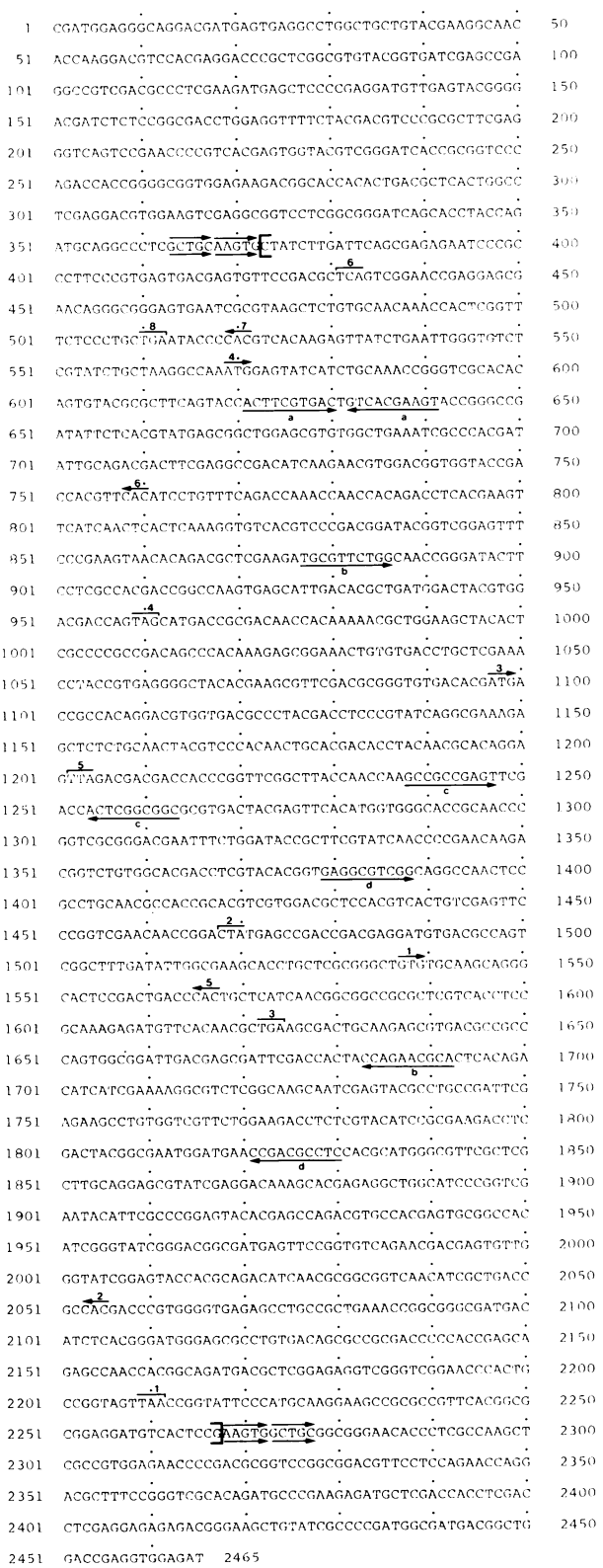

Fig. 3. Sequence of ISH1.8. Within the complete sequence of the 2465-bp fragment *Cla*I-10, the ends of ISH1.8 are indicated with brackets. The two 5-bp repeats flanking the element are shown within arrows. Several open reading frames are marked and numbered at their initiation and termination codons above the squence (1 – 6), as well as the initiation codon of a hypothetical reading frame reading out of the element and the termination codon of one reading into the element (7 and 8). The longest reading frame (1) begins at nucleotide 1165 and ends at 1863. Four internal inverted repeats of 10 bp are indicated by arrows below the sequence (a – d).

ment containing the point of circularization of pΦHL. The *Xho*I site is located immediately to the left of ISH1.8 in position B, the *Sal*I site to the right of ISH1.8 in position A (see Figure 2).

112 bp of pΦHL originate from the two adjacent ISH1.8 elements. From the sequence of the region containing the point of circularization (Figure 5) we conclude that pΦHL was formed by recombination within a 9-bp sequence (TCCCGCCCT). This sequence is present in ISH1.8 as an inverted repeat separated by 50 bp. Phage genomes carrying two copies of ISH1.8 in inverted orientation therefore contain two direct repeats of this 9-bp sequence. The plasmid pΦHL was generated by recombination within one of these direct repeats (Figure 6). A possible stem-loop structure in pΦHL with a stem of 31 bp consisting of the ends of the two copies of ISH1.8 and a loop of 50 bp from the ISH1.8 in site B is illustrated in Figure 7.

Discussion

Deletion of ISH1.8

The generation of new phage variants, easily isolated from single plaques, is another manifestation of the high variability of genomes in *H. halobium* (Schnabel *et al.*, 1982b). The isolation of the new variants, ΦH7 and ΦH8 lacking ISH1.8 in position B, without applying any selection procedure indicates that deletion of ISH1.8 occurs frequently. Apparently, at least two types of deletions can be caused by the element: (i) exact or almost exact deletion of ISH1.8, as in ΦH8; (ii) deletion of ISH1.8 together with flanking sequence, as in ΦH7. Indicated by the loss of restriction sites, DNA on either side of the element was lost in this case. So far no phage variants lacking both copies of ISH1.8 have been isolated. Therefore, it cannot be excluded that at least one copy of ISH1.8 might be required for phage growth either in position A or in position B.

Sequence of ISH1.8

In contrast to other insertion sequences of *Halobacterium* (Simsek *et al.*, 1982; DasSarma *et al.*, 1983; Xu and Doolittle, 1983; Pfeifer *et al.*, 1984) ISH1.8 does not have the features typical of most insertion elements, i.e., it does not have an inverted repeat at its ends and it is not always flanked by a target site duplication. Only one other halobacterium element, ISH25, also lacks the typical structure, but in this case homologous recombination probably caused the observed translocation (Pfeifer *et al.*, 1984). Several possible explanations must be considered for ISH1.8.

(i) ISH1.8 is not a transposable element. This cannot be excluded, because transposition has so far not been directly observed. On the other hand, deletions associated with the loss of ISH1.8 resemble those generated by eubacterial and eukaryotic transposable elements (see e.g., Reif and Saedler, 1977; Habermann and Starlinger, 1982; Liebmann *et al.*, 1981) and the presence of the element in several copies in the genomes of phage and host are typical features of transposable elements. Insertion of ISH1.8 by homologous recombination is impossible, because no sequence homology was detected in the regions flanking the two copies of ISH1.8 in ΦH DNA.

(ii) ISH1.8 causes 5-bp duplications in the target site. The element in position A might lack the duplication because the event inserting it there was a co-integration event, or it could have lost the duplication, either by intramolecular transpositional deletion or, more unlikely, by point mutations.

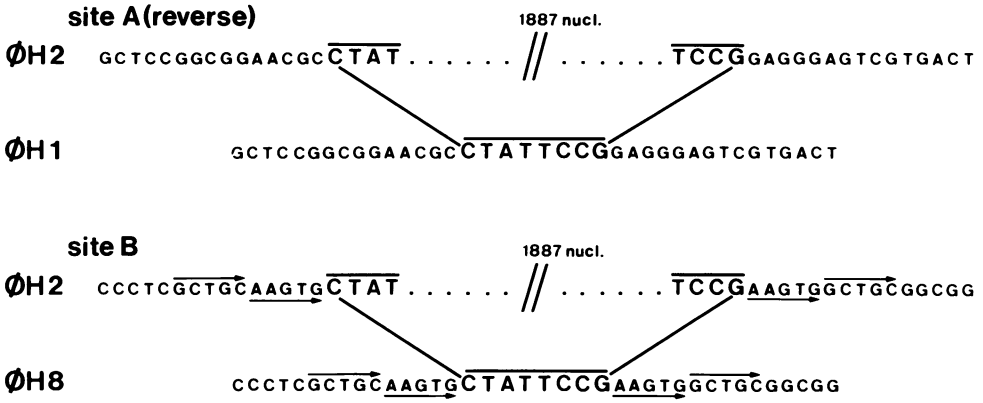

Fig. 4. Sequence of sites A and B lacking ISH1.8. The ends of both copies of ISH1.8 in ΦH2 DNA are compared with the empty sites. Site A was reversed to show ISH1.8 in the same orientation. Large letters denote nucleotides of ISH1.8 including the 8-bp sequence, composed of 4 bp from each end of the element, which is present in both sites lacking ISH1.8. Small letters are flanking sequence. The two direct repeats of 5 bp which lie adjacent to the element in site B are still present after the deletion (arrows above and below the sequence).

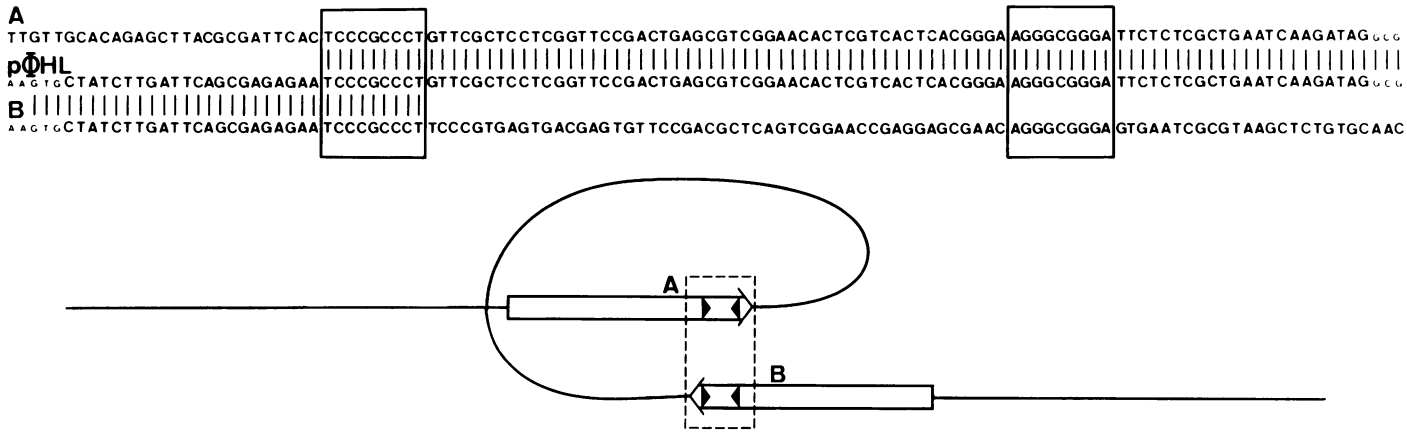

Fig. 5. Circularization point of the plasmid pΦHL. The sequence of the plasmid pΦHL (**middle**) is compared with the right end of ISH1.8 in site A (**above**) and to the left end of ISH1.8 in site B (**below**). The drawing below illustrates the situation in ΦH2; the dotted box represents the parts shown in the sequence above. Large letters denote nucleotides of ISH1.8. Arrowheads in the drawing and boxes in the sequence represent the 9-bp inverted repeats.

(ii) ISH1.8 inserts by a mechanism different from that of other transposable elements. There has recently been a report on Tn554 from *Staphylococcus aureus*, a transposable element which also lacks an inverted repeat and a target site duplication, but clearly transposes (Murphy and Löfdahl, 1984).

Sequence of empty sites

It is not clear whether ΦH1, which lacks ISH1.8 in site A, is derived from ΦH2 by deletion or whether it represents the original site before insertion of ISH1.8. However, ΦH8, was clearly obtained from ΦH2 by deletion of the element from site B. The presence of the same 8-bp sequence, 4 bp from each end of the element, may therefore be interpreted in two different ways. It could indicate that ΦH1 also originated by deletion from ΦH2, leaving 8 bp of the element behind as in the other case. Alternatively, it could mean that this 8-bp sequence is not part of the element, but rather a specific recognition sequence for insertion of ISH1.8, which would then have a size of 1887 bp. Then, the structure of ΦH8 DNA would be due to an exact deletion, restoring the original sequence and ΦH1 DNA could either represent the original structure or also be caused by exact deletion.

Deletions associated with eubacterial IS-elements are either deletions of flanking DNA only, probably caused by intramolecular transposition (see e.g., Saedler *et al.*, 1980), or deletions of the element itself, with or without flanking DNA, which always involve short direct repeats (see e.g., Sommer *et al.*, 1981). Short direct repeats have also been shown to be important in the formation of spontaneous deletions not involving IS-elements (Farabaugh *et al.*, 1978; Albertini *et al.*, 1982). No such repeat which could be associated with the deletion of ISH1.8 was found; the existing 5-bp repeats apparently have not been used, because they are still present after the deletion.

A 9-bp repeat involved in formation of pΦHL

As illustrated in Figure 6, recombination to generate the plasmid pΦHL from the phage genome takes place within the 9-bp sequence TCCCGCCCT, present as an inverted repeat in ISH1.8 and therefore as two direct repeats in phage genomes containing two copies of ISH1.8. Within ISH1.8, there are four sequences present as perfect inverted repeats of 10 bp and 10 sequences present as inverted repeats of 9 bp. All of these are also direct repeats in genomes containing two copies of ISH1.8. If the frequent circularization only occurs at this 9-bp sequence, it must involve a site-specific mechanism.

Materials and methods

Materials

[α-^{32}S]dATP was from Amersham, *Escherichia coli* DNA polymerase I large

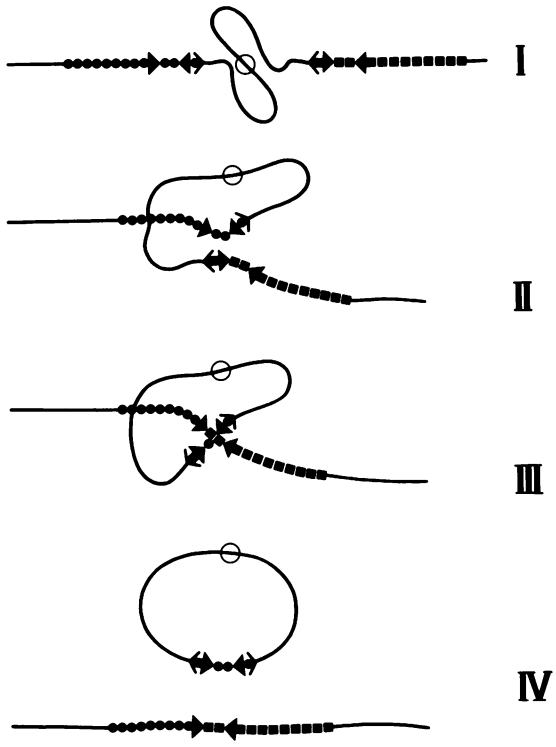

Fig. 6. Model for the release of the plasmid pΦHL from the phage genome. (Not drawn to scale.) Part of a phage genome containing two identical inverted copies of ISH1.8 flanking the 12-kb L segment is shown on top (**I**). The circle symbolizes the origin of replication. Each ISH1.8 contains an inverted repeat of 9 bp (black arrowheads); the genome therefore contains two direct repeats of this sequence. A double-strand exchange within one of these repeats (**II**) would lead to the release of the plasmid pΦL (**III**). If the only replication origin of the phage lies on the L segment, pΦHL will be able to replicate, whereas the remaining phage genome will be lost.

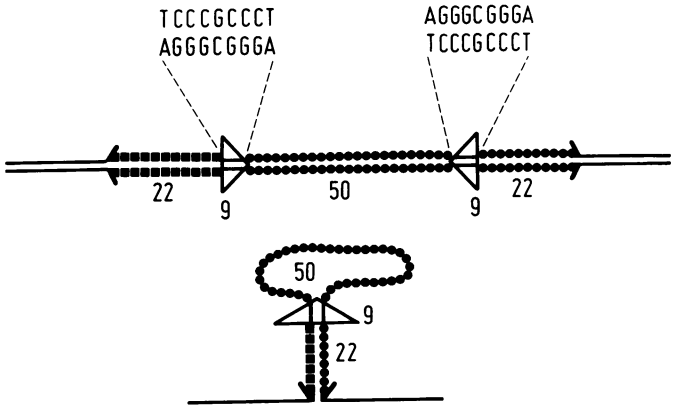

Fig. 7. Possible stem-loop structure in the plasmid pΦHL. 112 bp of the plasmid pΦHL originate from the two copies of ISH1.8 which flank the L segment in the phage genome. This 112-bp region contains an inverted repeat of 31 bp, composed of the ends of the two ISH1.8 elements and including the 9-bp sequence TCCCGCCCT which is involved in the release of pΦHL from the phage genome, as illustrated in Figure 6. This inverted repeat could form a stem of 31 bp with a loop of 50 bp originating from one ISH1.8.

fragment (Klenow fragment) was from Bethesda Research Laboratories ('ultra pure' quality), T4 DNA ligase was from New England Nuclear. *E. coli* strain GM33 (dam$^-$) was obtained from Dietmar Kamp.

Phase ΦH variants used are ΦH1, the predominant variant of the original mixture (Schnabel *et al.*, 1982a), ΦH2 (Schnabel *et al.*, 1982b), ΦH7 and ΦH8, which were isolated from single plaques as described in Results. Growth of phage ΦH, purification of phage and of phage DNA, and agarose gel electrophoresis of DNA were as described previously (Schnabel *et al.*, 1982a, 1982b; Schnabel and Zillig, 1984).

Cloning

Restriction fragments shown in Figure 2 were cloned into pBR322. As several *Cla*I restriction cleavage sites in ΦH DNA are lost after cloning of fragments into *E. coli* vectors due to *dam* methylation, plasmids containing *Cla*I-fragments were grown in a dam$^-$ host (GM33).

Sequencing

All sequencing was performed by the chain termination method (Sanger *et al.*, 1977, 1980) using the M13 cloning and sequencing technique (Messing and Vieira, 1982) except for a region of severe compression which was confirmed by sequencing according to Maxam and Gilbert (1980). Computer-aided editing and comparisons of sequences were done using the programs of Devereux *et al.* (1984) and Staden (1980).

Complete sequence of ClaI-10 containing ISH1.8 at position B. (For an illustration of the restriction sites, see Figure 2.) The isolated fragment *Cla*I-10 of ΦH1 DNA was circularized by ligation. Sonication of this DNA, cloning of the fragments into the *Sma*I site of M13mp8 and sequencing were done according to Deininger (1983) following a protocol of Amersham International plc.

Sequencing of the ends of ISH1.8 in position A. A 2.4-kb *Sal*I fragment containing ISH1.8 was isolated from a clone containing a 3.7-kb *Bam*HI fragment of ΦH2 DNA (see Figure 2). After cleavage with *Hpa*I, which has a single cut within ISH1.8 close to one end, the two *Sal*I/*Hpa*I fragments were cloned in both directions into M13 using *Sal*I/*Sma*I double cleavages of the vectors M13mp8 and M13mp9, and the sequence of the 157-bp *Sal*I/*Hpa*I fragment containing one end of ISH1.8 was determined in both directions. The sequence of the large *Sal*I/*Hpa*I fragment from the *Sal*I end was determined in order to obtain the other end of ISH1.8. This sequence was confirmed by sequencing *Alu*I fragments of the 2.4-kb *Sal*I fragment cloned into the *Sma*I site of M13mp9.

Sequencing of the sites lacking ISH1.8. A 214-bp *Xho*I fragment containing site B without ISH1.8 (see Figure 2) was sequenced by cloning *Xho*I fragments of a 2-kb *Pst*I fragment containing site B into *Sal*I cleaved M13mp8. A 310-bp *Sal*I fragment containing site A without ISH1.8 was isolated from *Bam*HI-13 (see Figure 2) and either inserted into *Sal*I-cleaved M13mp8 or cleaved with *Alu*I and then inserted into *Sal*I/*Sma*I-cleaved M13mp9 in order to sequence both strands.

Sequence of the region containing the point of circularization of plasmid pΦHL. A 500-bp fragment containing the region of interest was isolated after double cleavage of a plasmid carrying a *Bam*HI fragment containing the circularization point with *Bam*HI and *Xho*I. Of this, ~400 bp from *Xho*I were sequenced after inserting either the 500-bp fragment into *Sal*I/*Sma*I-cleaved M13mp8 or *Alu*I fragments of it into *Bam*HI/*Sma*I-cleaved M13mp9.

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