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Insertion Elements and Deletion Formation in a Halophilic Archaebacterium

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Deletion events that occur spontaneously in 36-kilobase-pair (kbp) plasmid pHH4 from the archaebacterium *Halobacterium halobium* were investigated. Four different deletion derivatives with sizes ranging from 5.7 to 17 kbp were isolated. Three of these deletion variants derived from pHH4 (pHH6 [17 kbp], pHH7 [16 kbp], and pHH8 [6.3 kbp]), whereas the 5.7-kbp plasmid pHH9 derived from pHH6. Strains containing pHH6, pHH7, or pHH9 each lacked the parental plasmid pHH4, while pHH8 occurred at a 1:1 ratio together with pHH4. Common to all of these plasmids was the 5.7-kbp region of pHH9 DNA. The regions containing the fusion site in the deletion derivatives were investigated and compared with the corresponding area of the parental plasmid. Each deletion occurred exactly at the terminus of an insertion element. In pHH6 and pHH7, a halobacterial insertion element (ISH2) was located at the deletion site. The DNA fused to ISH2 displayed a 7-base-pair (bp) (pHH7) or 10-bp (pHH6) sequence homology to the inverted repeat of ISH2. In the two smaller plasmids, pHH8 and pHH9, an ISH27 element was located at the deletion site. Most likely, all of these smaller plasmids resulted from an intramolecular transposition event. The ISH27 insertion sequence contains a 16-bp terminal inverted repeat and duplicates 5 bp of target DNA during the transposition with the specificity 5'ANNNT3'. Four ISH27 copies were analyzed, and two ISH27 element types were identified that have approximately 85% sequence similarity. The ISH27 insertion elements constitute a family which is related to the ISH51 family characterized for *H. volcanii*, another halophilic archaebacterium.

The plasmid DNA of the extremely halophilic archaebacterium *Halobacterium halobium* undergoes frequent alterations due to the activities of various insertion elements. Insertions as well as deletions occur spontaneously at frequencies as high as 10^{-2}. Both mutation types were found for the p-*vac* gene, which encodes a gas vacuole protein located on 150-kilobase-pair (kbp) plasmid pHH1 (7). pHH1 and a 36-kbp deletion derivative (pHH4) found in a p-*vac*-negative mutant gave rise to further deletion variants. With pHH4 as the parental plasmid, deletion derivatives with sizes of 20 to 5.7 kbp occur at high frequency during development of a colony (13). The deletion plasmids can be visualized by using a defined DNA fragment that presumably contains the functions necessary for pHH1 replication as a probe in Southern analysis. After an enrichment procedure, the resultant smaller plasmids can be isolated (13; this report). Plasmids pHH6 (17 kbp), pHH7 (16 kbp), and pHH8 (6.3 kbp) were obtained from pHH4, and pHH9 (5.7 kbp) was isolated as a deletion variant of pHH6 (13; this report). The high frequency of deletion formation within the pHH1-type plasmids could be due to the action of the multiple insertion elements found in *H. halobium*. Eight different halobacterial insertion elements (ISH elements) in *H. halobium* have been characterized; except for ISH*1.8*, all of them were isolated from the bacterio-opsin (*bop*) gene region of Bop mutants (14, 15). Most of these ISH elements are also found in the plasmid DNA (12).

Only two deletion events have been previously investigated in halobacteria. One was described for bacteriophage ϕH2 DNA, in which recombination between two ISH*1.8* elements leads to formation of a 12-kbp plasmid (18). Another deletion event was characterized that affects the upstream region of the *bop* gene in Bop mutant M86, in which recombination between 8-base-pair (bp) repeats leads to deletion of the intervening 1,883 bp of DNA (9).

In this study, we analyzed the deletion formation in *H. halobium* that occurs within pHH1-type plasmids. Regions involved in deletion formation were investigated by nucleotide sequence determination in the parental plasmids as well as in the deletion derivatives. The analysis strongly suggests the involvement of active transposition of insertion elements.

MATERIALS AND METHODS

Materials. [^{35}S]dATP and [^{32}P]dATP were obtained from Amersham Corp. Restriction endonucleases and the Klenow fragment of DNA polymerase I were from Bethesda Research Laboratories, Inc., or Boehringer Mannheim Biochemicals. Nylon membranes for Southern transfer were purchased from Pall Biodyne. T7 DNA polymerase was obtained with the Sequenase sequencing reagents from U.S. Biochemicals. The Geneclean kit was obtained from Bio101.

Methods. (i) Strains of halobacteria and DNA isolation. *H. halobium*(pHH4) is a derivative of *H. halobium* DSM671, a Vac$^-$ strain (16). Isolation of pHH4 deletion derivatives and analysis of the plasmid DNA were done as previously described (13). Covalently closed circular DNA was purified by CsCl-ethidium bromide gradient centrifugation (13).

(ii) Characterization of plasmids. Plasmid DNA was analyzed on 1% agarose gels in Tris-phosphate buffer (16). Subfragments of pHH4 and fragments containing the fusion sites were isolated from agarose gels by using the Geneclean procedure as recommended by the manufacturer. pHH6 and pHH7 were cloned as single *Pst*I fragments into pKG2 (8), whereas pHH8 and pHH9 were gel purified and cloned as single *Eco*RI fragments into pUN121 (10). The 4.2-kbp *Bam*HI (pHH4), 2.0-kbp *Cla*I (pHH4), and two *Pst*I-*Cla*I fragments (pHH6 and pHH7) containing the fusion sites were cloned into pUC18 (21) for further analysis. A 0.8-kbp

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*Cla*I-*Eco*RI fragment of pHH8 and a 1.4-kbp *Sph*I-*Eco*RI fragment of pHH9 were used for determination of the fusion site in the smaller plasmids. Nucleotide sequence determination was done by the procedure of Sanger et al. (17) by using the Sequenase system. For determination of ISH2 and adjacent DNA sequences, two synthetic oligonucleotides (5'CAAACAGACGCGAGAGCT3' and 5'TCGTCTTATTT CGATTT3') were used as primers for the sequencing reaction. Synthetic oligonucleotides 5'AGAAGCTGGTCCTCG TG3' and 5'CAACGGGCAGCGTGGAC3' were used for determination of ISH27 and adjacent nucleotide sequences. Computer-aided editing was done with the program of Staden (20). Radioactive labeling of fragments for Southern analysis was done by using random primers as described by Feinberg and Vogelstein (4). DNA fragments separated on 1% agarose or 5% polyacrylamide gels were transferred onto nylon membranes as described by Southern (19).

RESULTS

Isolation of deletion derivatives. The spontaneous deletion derivatives pHH6 (17 kbp), pHH7 (16 kbp), and pHH8 (6.3 kbp) could be recovered as the major plasmids from *H. halobium* cells with no residual pHH4. In contrast, the presence of derivative pHH8 was not accompanied by complete loss of the parental pHH4 plasmid. After an enrichment procedure for pHH8, each single colony contained pHH4 and pHH8 at a 1:1 ratio.

After a similar screening procedure (13), cultures containing pHH6 were found to produce a 5.7-kbp derivative, pHH9. pHH9 was present in *H. halobium* cells with no parental pHH6. Southern analysis of a DNA preparation from a pHH9-carrying strain probed with a fragment containing the pHH1 origin of replication (13) indicated a 4.5-kbp covalently closed circular DNA. The small variant was, however, never detectable in any further screening study (data not shown).

The four deletion derivatives pHH6 through pHH9 were compared by restriction endonuclease digestion and hybridization to the respective parental plasmid. Restriction maps of these plasmids in comparison with pHH4 are shown in Fig. 1. All four plasmids shared a common 5.7-kbp region.

ISH2 involvement in the formation of pHH6 and pHH7. Comparison of pHH6 and pHH7 with pHH4 DNA indicated that they contained almost identical pHH4 sequences. A size difference in restriction endonuclease fragments was found in two cases. Hybridization experiments using an ISH27-specific probe derived from the *bop* gene area in Bop mutant O5 (14) indicated that the 4.1-kbp *Cla*I fragment in pHH6 incurred an additional ISH27 element (data not shown). This was verified by DNA sequence determination (see below). Interestingly, the *Pst*I-*Cla*I fragments containing the fusion site of plasmids pHH6 and pHH7 also differed (Fig. 2a). pHH7 contained more DNA of the 2.0-kbp *Cla*I fragment than did pHH6, suggesting that the deletion event leading to the two plasmids occurred at different sites (Fig. 2a).

To analyze the possible involvement of the ISH2 element in deletion formation, we investigated the presence of ISH2 in both deletion variants. Southern analysis of the restriction endonuclease-digested wild-type fragment and the corresponding fusion fragments of the deletion variants probed with an ISH2 copy (15) indicated that the sizes of internal ISH2 fragments and fragments containing the left terminus of the element were identical in all three cases. In contrast, fragments containing the right terminus of ISH2 were different in size (data not shown). This was verified by DNA

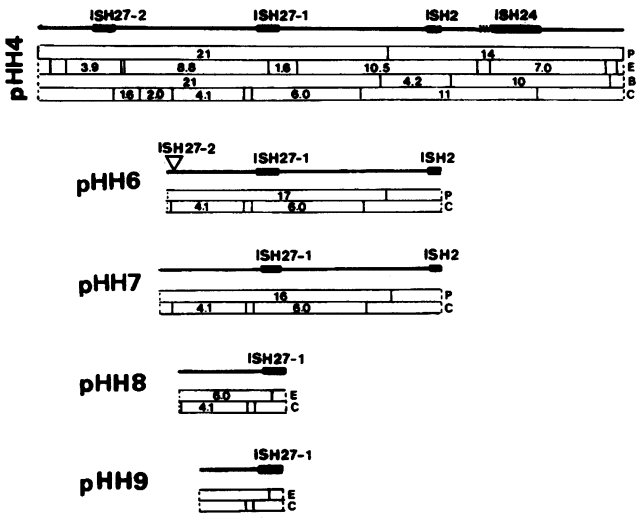

FIG. 1. Restriction maps of pHH4 and the four derivatives investigated. The restriction endonucleases used were *Pst*I (P), *Eco*RI (E), *Bam*HI (B), and *Cla*I (C). The numbers indicate fragment sizes in kilobase pairs. The locations of various insertion elements within pHH4 and its derivatives are indicated by black boxes. The integration site of the ISH27-2 element in pHH6 is indicated by a triangle.

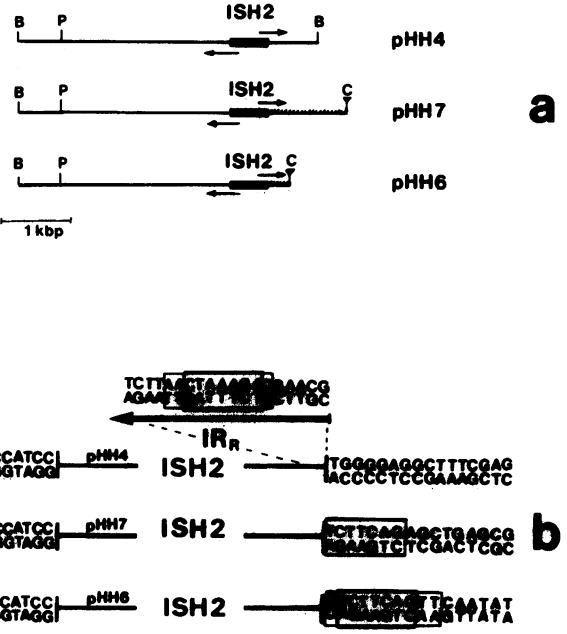

FIG. 2. Characterization of the fusion region in pHH6 and pHH7. (a) Comparison of the *Bam*HI-*Cla*I fragments of pHH6 and pHH7 with the 4.2-kbp *Bam*HI fragment of pHH4. The location of ISH2 is indicated together with the directions (arrows) of the oligonucleotide primers used for DNA sequence determination. The hatched regions in the fusion fragments of pHH6 and pHH7 correspond to the DNA derived from the 2.0-kbp *Cla*I fragment of pHH4. Restriction endonuclease sites are indicated as B (*Bam*HI), P (*Pst*I), and C (*Cla*I). (b) DNA sequence determination of the regions surrounding the ISH2 elements in pHH4, pHH6, and pHH7. The DNA sequence of one 19-bp inverted repeat (IR_R) is given at the top. The boxed regions depict similarities between the inverted repeat of ISH2 and the fused DNA sequences.

sequence determination using synthetic oligonucleotides as primers (see Materials and Methods). As expected, the DNA sequences obtained for the left ISH2 terminus and adjacent DNA were identical in all three cases (Fig. 2b). The DNA sequences determined for the internal ISH2 sequences at the right termini were identical; however, the nucleotide sequence immediately adjacent to the ISH2 element was different in each case (Fig. 2b). Thus, the deletion event leading to pHH6 and pHH7 occurred exactly at the terminus of ISH2.

The DNA sequences adjacent to the ISH2 elements in pHH6 and pHH7 were derived from the 2.0-kbp *Cla*I fragment of pHH4. Fusion occurred at two different locations, 1.2 kbp (pHH7) and 0.3 kbp (pHH6), within this fragment. Comparison of the nucleotide sequence fused to ISH2 indicated a common 7-bp (5'TCTTCAG3') DNA sequence. A similar sequence (with a 1-bp mismatch) was found in the opposite orientation within the inverted repeat sequence of ISH2 (Fig. 2b). In pHH6, the short homologous sequence between the fused DNA and the inverted repeat of ISH2 was 10 bp long (Fig. 2b).

The two DNA sequences found adjacent to the ISH2 termini in pHH4 indicated no direct repeat. Upon transposition, ISH2 usually duplicates target DNAs of various lengths (Table 1). Investigation of ISH2 integration sites in the *bop* gene region of Bop mutants and in the p-*vac* gene region of Vac mutants indicated that ISH2 elements duplicate target DNAs with sizes of 10, 11, 12, or 20 bp (Table 1). Comparison of these target DNAs showed no specificity of target DNA recognition for ISH2.

The absence of a direct sequence repeat surrounding the ISH2 element in pHH4 may be related to the fact that this ISH element was involved in an earlier deletion event. Indeed, this region of pHH4 represents a fusion between a sequence originally derived from the pHH1 *Pst*I fragment, P2, and another DNA (PX; see below).

Comparison of the internal ISH2 DNA sequence indicated a difference from the published ISH2 sequence (3). We found an additional adenosine residue at position 388 of the ISH2 nucleotide sequence (the sequence reads 5'GTGCAC GAACT3' instead of 5'GTGCACGACT3'). The additional adenosine residue was present in each ISH2 element we investigated (Table 1).

ISH27 involvement in the formation of pHH8 and pHH9. The deletion events leading to the two smaller plasmids pHH8 and pHH9 each occurred in the 1.6-kbp *Eco*RI fragment of pHH4 (Fig. 1). This fragment, as well as the adjacent 8.8-kbp *Eco*RI fragment, hybridized with the ISH27 insertion element isolated from Bop mutant O5 (14). To analyze the possible involvement of the ISH27 element in the formation of pHH8 and pHH9, the terminal structural features of the ISH27 elements found in pHH4, pHH6, and Bop mutant O5 were determined by DNA sequence analysis. In each case, ISH27 contained a 16 (or 17)-bp terminal inverted repeat with 1-bp mismatch (Fig. 3A). The 5 bp of duplicated target DNA always exhibited the same feature; the element was flanked by the sequence 5'ANNNT3'. This sequence arrangement made it difficult to determine whether the target site duplication is 5 bp (5'ANNNT3') in a 16-bp terminal inverted repeat of ISH27 or 3 bp (5'NNN3') with a 17-bp terminal inverted repeat of the ISH27 element (Fig. 3A). If the thymidine residue (and the adenosine residue at the other terminus) is part of the ISH27 element, the duplication is 5'NNN3'. In this case, an adenosine residue always occurs 5' to the left target site duplication together with a thymidine residue 3' to the target DNA duplication at the right terminus of the element; i.e., ISH27 transposition requires specific DNA recognition outside the target DNA (Fig. 3A). We assume that 5'ANNNT3' represents the target DNA duplication of ISH27.

TABLE 1. Duplicated target DNA of insertion element ISH2[a]

Mutant	Target site	Length (bp)
IV-3	TCCAGGGCGT AGGTCCCGCA	10
W11	ACGGGAAGAC TGCCCTTCTG	10
W1	GTGCGAACGG CACGCTTGCC	10
SD19	CGGCTCCGTG GCCGAGGCAC	10
L33	TGGCCTCACA ACCGGAGTGT	10
E7	CTCGTTCGCT GAGCAAGCGA	10
I-1	TGCCTCCGAG ACGGAGGCTC	10
M18, M89	ACCCAACAGGT TGGGTTGTCCA	11
IV-12	GAATACACACG CTTATGTGTGC	11
I-5	ACCCAGCCCCG TGGGTCGGGGC	11
III-1, III-3	GTCCGGGTACAG CAGGCCCATGTC	12
R1mR	ACCCCATCTACTGGGCGCGG TGGGGTAGATGACCCGCGCC	20

[a] The ISH2 integrations in mutants IV-3, IV-12, M18, M89 (12,15), W11, W1 (14), SD19, L33, and R1mR (3) are found in or near the *bop* gene. The ISH2 element in E7 is found in pHH1 close to the p-*vac* gene, as are the target sites of ISH2 integrations in Vac mutants III-1, III-3, I-1, and I-5 (unpublished data).

To determine the site of the deletion leading to pHH8, a 0.8-kbp *Cla*I-*Eco*RI fragment containing the fusion site was isolated. This fragment contained DNAs from the 1.6-kbp *Eco*RI and 2.0-kbp *Cla*I fragments of pHH4. Comparison of the nucleotide sequences determined for these pHH4 fragments and for the pHH8 fusion region indicated that the deletion occurred exactly at the terminus of the ISH27 element (Fig. 3B). The DNA sequences fused to ISH27 (5'AGACT3') exhibited no homology to the terminal inverted repeat of the element but displayed the characteristics of an ISH27 target site duplication (5'ANNNT3'; Fig. 3B).

Analysis of pHH6 deletion derivative pHH9 indicated that the fusion occurred between DNA sequences found in the 1.6-kbp *Eco*RI fragment and in the 4.1-kbp *Cla*I fragment of pHH6. A synthetic oligonucleotide complementary to an internal ISH27 sequence was used as a primer to determine the nucleotide sequence across the fusion region in pHH9. As in plasmid pHH8, the deletion occurred exactly at the terminus of ISH27 (Fig. 3B). The DNA sequences fused to ISH27 (5'AAAAT3') also showed the structural feature of ISH27 target DNA duplications.

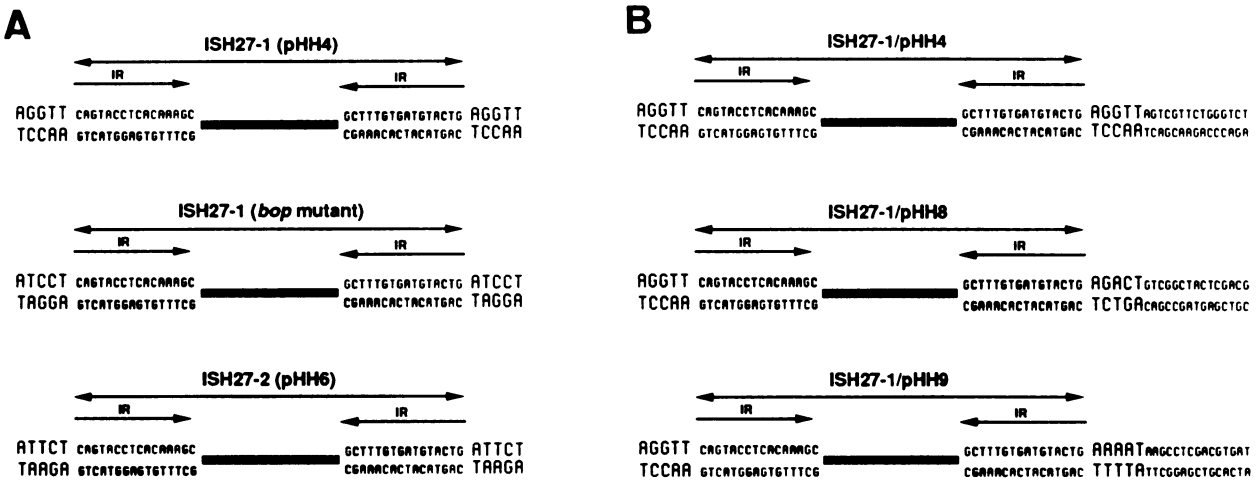

FIG. 3. Target DNA duplications and inverted repeats (IR) of ISH*27* elements. (A) Duplicated target DNA of the ISH*27* element found in pHH4 (ISH*27-1*), in the *bop* gene region (ISH*27-1*), and within pHH6 (ISH*27-2*). The integration of ISH*27* in Bop mutant O5 occurred 21 bp upstream of the ATG start codon of the *bop* gene. (B) DNA sequences found adjacent to ISH*27-1* in the fusion regions of pHH8 and pHH9 in comparison with those next to ISH*27-1* in parental plasmid pHH4.

pHH4 is a deletion derivative of a larger plasmid. The 36-kbp plasmid pHH4 was isolated as a deletion derivative of the 75-kbp plasmid pHH3 found originally in a purified colony of the *H. halobium* Vac$^-$ strain DSM671 of the German strain collection (13, 16). Our *H. halobium* wild-type strain contains the 150-kbp plasmid pHH1 as the major plasmid species. Previously, pHH1 seemed to be the parental plasmid of pHH3 because of its strong DNA sequence homology to pHH3 and its larger size (16). Comparative investigation of pHH3 and pHH1, however, indicated the presence of about 6 kbp of DNA in pHH3 and pHH4 (PX; Fig. 4) that was present in neither pHH1 nor the total DNA of our *H. halobium* wild-type strain (data not shown). All other pHH3 and pHH4 regions were present in pHH1 (Fig. 4). Thus, plasmid pHH4 contains two noncontiguous pHH1 regions together with a short region (PX) of unknown origin. Fusions occurred between DNA sequences derived from pHH1 *Pst*I fragments P7 and P1 (A), between P1 and PX DNAs (B), and between PX and sequences derived from pHH1 *Pst*I fragment P2 (C; Fig. 4).

A closer investigation of the DNA sequences across the fusion region (A) revealed the presence of a second ISH*27* element in pHH4. Preliminary DNA sequence determination of this ISH*27* element together with restriction endonuclease analysis revealed an ISH*27* element with extended sequence similarity to the additional ISH*27* element in pHH6 but with approximately 85% DNA sequence similarity to the other ISH*27* element in pHH4 and to the ISH*27* element of Bop mutant O5 (data not shown). The terminal inverted repeats of all four elements were identical. Thus, there are at least two types of ISH*27* elements. These two were termed ISH*27-1* and ISH*27-2* (Fig. 4).

No target site duplication was found with the ISH*27-2* element near the fusion site (A) in pHH4 (Fig. 5A). The DNA sequences up- and downstream of the element (5′AGACT3′ and 5′ATTCT3′) had the 5′ANNNT3′ sequence characteristic. The deletion event in pHH3 leading to the fusion of P7 with P1 DNA sequences in pHH4 occurred exactly at the terminus of the ISH*27-2* element (Fig. 5A).

The region (B [Fig. 4]) containing the transition to the

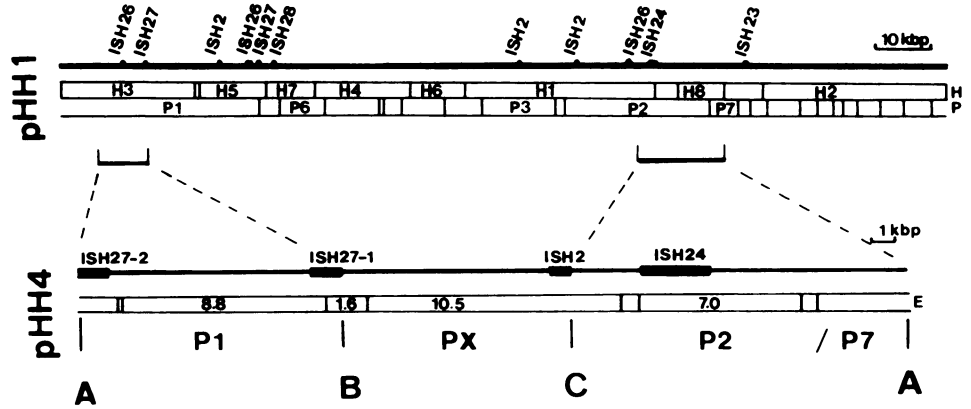

FIG. 4. Comparison of plasmids pHH1 and pHH4. A restriction map for pHH1 is given for *Hin*dIII (H) and *Pst*I (P) at the top, and the *Eco*RI (E) map of pHH4 is at the bottom. The numbers in the pHH1 map indicate *Hin*dIII and *Pst*I fragments described by Pfeifer et al. (16). The numbers in the pHH4 restriction map are fragment sizes in kilobase pairs. The origin of pHH4 regions from pHH1 *Pst*I fragments is indicated. The PX region is not found in pHH1. The fusions between these regions are labeled A through C. The DNA sequence homology between PX and pHH1 *Pst*I fragment P3 as described in reference 13 is due to an ISH*2* element found in both DNAs.

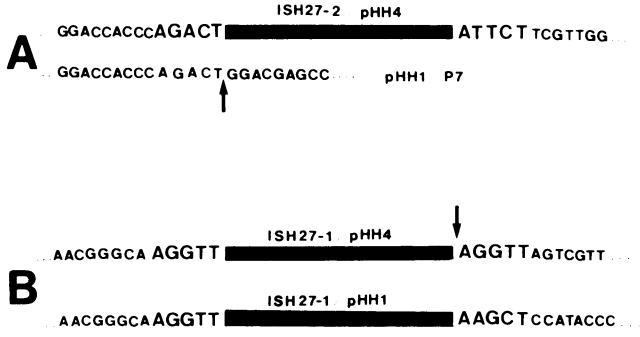

FIG. 5. DNA sequences of the fusion regions in pHH4. (A) The DNA sequence in fusion A of pHH4 (top) is compared with the corresponding pHH1 sequence of *Pst*I fragment P7 (bottom). The site of the deletion in pHH1 fragment P7 is marked by an arrow. (B) The DNA sequence found adjacent to the ISH*27-1* element in pHH4 (top) is compared with the corresponding sequence of the same element found in pHH1 (bottom). The site of the deletion that occurred in the DNA sequence of hypothetical plasmid pHH0 is marked in the pHH4 sequence by an arrow.

DNA sequence PX was located next to the ISH*27-1* element. Surprisingly, this insertion element was integrated between direct repeats of target DNA (Fig. 5B). Analysis of the corresponding ISH*27* element in plasmid pHH1, however, indicated no direct repeat (Fig. 5B). The DNA sequences adjacent to ISH*27-1* in pHH4 were not present in the pHH1-containing strain (data not shown). We conclude that a deletion mediated by ISH*27-1* occurred in a pHH1 precursor plasmid (pHH0) encompassing the PX DNA sequence still present in pHH4. This deletion event must have occurred after single-colony separation of the first *H. halobium* isolate containing this hypothetical pHH1 precursor. Two other alterations distinguish pHH1 from plasmid pHH4: the ISH*26* element found in pHH1 *Pst*I fragment P1 is not present in the corresponding region of pHH4, and the ISH*27-2* element of pHH4 is absent in pHH1 (Fig. 4). Thus, the *H. halobium* strains propagated from various strain collections all contain pHH1-type plasmids (e.g., pHH1, pHH3, and pHH4) that are already deletion products of a larger precursor plasmid and subsequently incurred additional insertion elements at various places. A pedigree of the different *H. halobium* plasmids is shown in Fig. 6.

The fusion region (C [Fig. 4]) in plasmid pHH4 is next to the ISH*2* element involved in the deletions which produced pHH6 and pHH7.

DISCUSSION

The variability found with pHH1-type plasmids in various *H. halobium* strains resembles the differences found in plasmids of the closely related halobacterial species *H. cutirubrum* and *H. salinarium* (16; unpublished data). Insertion and deletion events occurred independently in each strain during propagation, leading to different although highly similar pHH1-type plasmids. Our results demonstrate that the plasmid variability can be traced back to the action of insertion elements. In each case, a copy of an ISH element was found adjacent to the deletion site. The deletion event leading to plasmids pHH6 (17 kbp) and pHH7 (16 kbp) occurred exactly at the terminus of an ISH*2* element, and the DNA sequences fused to ISH*2* in these two cases exhibited similarities to sequences present in the terminal inverted repeat of ISH*2*. This same ISH*2* element of pHH4 was most likely involved in a deletion event that generated pHH4.

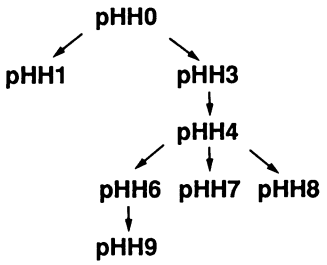

FIG. 6. Pedigree of the pHH1-type plasmids of *H. halobium*. pHH0 is the hypothetical precursor plasmid of pHH1 and pHH3.

Plasmids pHH8 (6.3 kbp) and pHH9 (5.7 kbp) each contained an ISH*27* element adjacent to the fusion site. No DNA sequence homology was found between the DNA fused to the element and the inverted repeat of ISH*27*. However, we observed similarities between the fusion regions and the 5'ANNNT3' characteristic of the duplicated target DNA found for ISH*27* transpositions. Three ISH*27* transposition events were investigated; in each, the motif 5'ANNNT3' occurred as the target site duplication. The same feature was observed in the DNA fused to ISH*27* in the deletion variants. The recognition of similar DNA sequences suggests that the short DNA sequence is used as a target site during replicative transposition of the ISH*27-1* element within pHH4. This intramolecular transposition led to the formation of two circular DNA molecules instead of one molecule containing two ISH*27-1* elements. Only the DNA species containing the origin of plasmid replication is maintained, while the other product is lost. The mechanism of deletion formation during intramolecular transposition has been described for several *Escherichia coli* transposable elements (2, 11).

The ability of ISH*27* to transpose is also demonstrated by the fact that during the course of enrichment for the smaller plasmids, several pHH4 plasmids incurred an additional ISH*27* element (13). Preliminary DNA sequence determination of these additional ISH*27* elements showed that they are identical to neither ISH*27-1* nor ISH*27-2*, indicating that they resulted from intermolecular transposition (unpublished data).

In pHH6 and pHH7, deletion formation could also be due to an intramolecular transposition event of ISH*2*. The target site duplications of ISH*2* elements indicate no specificity; thus, the involvement of active transposition cannot be proven. The DNA sequence homology observed between the direct repeat of ISH*2* and the DNA fused to the element was observed in only two of three deletion events and in none of the ISH*2* insertional events (Fig. 2b; Table 1). In pHH6 and pHH7, this DNA sequence homology might have provided additional information for target site recognition by the transposase. Structural similarities between the inverted repeat of the transposable element and the target site were described for integration hot spots of *E. coli* element Tn*10* (5).

The deletion events analyzed in *H. halobium* plasmids during these studies are different from deletions analyzed previously in *H. halobium*. The deletion in Bop mutant M86 was presumably generated by recombination between 8-bp repeats (9) without an insertion element. Such microhomologous recombination is often found as a mechanism for deletion formation in the absence of insertion elements (1). The deletion event described for halobacterial phage ϕH involves the action of two copies of insertion element

ISH*1.8*; recombination between a 9-bp repeat found within the two ISH*1.8* elements present in phage ϕH2 (rather than transposition of ISH*1.8*) leads to formation of a 12-kbp plasmid (18). In contrast, recombination between short sequence identities of two ISH elements of the same type has not been found for pHH1-type plasmids. Both pHH4 and pHH6 contain two ISH*27* copies. Although these copies have only approximately 85% sequence similarity, they contain DNA sequences that could be used in homologous recombination. The region containing the origin of plasmid replication is located in the intervening 9-kbp DNA (pHH4) or 6-kbp DNA (pHH6). None of the smaller plasmids resulted from homologous recombination between the two ISH*27* elements, and the frequency of deletion formation in strains containing plasmids with additional copies of ISH*27* was not enhanced (unpublished data).

The array of deletion variants analyzed in these studies allows the assignment of the region containing the origin of replication of the pHH1 plasmid. DNA sequence determination, together with transcript analysis and functional tests by transformation, will allow us to define and characterize this plasmid replication region in more detail. The reason why plasmid pHH9 was maintained in *H. halobium* cells with no residual pHH6 whereas the larger plasmid pHH8 could not be maintained is not understood. It is possible that pHH8 contains a mutation in a gene necessary for DNA replication in *trans* that must be supplied by parental plasmid pHH4. Another explanation for the coexistence of both plasmids could be that a gene needed for plasmid incompatibility is mutated. Further analysis is necessary to determine the difference between the two plasmids.

Four copies of the ISH*27* element were investigated, and all contained identical 16-bp terminal inverted repeat sequences. Preliminary DNA sequence determinations revealed two types of ISH*27* elements with approximately 85% sequence similarity (data not shown). Comparison of the total DNA sequences of the different ISH*27* elements will assist in determining regions that are conserved and perhaps necessary for ISH*27* transposition. The terminal inverted repeat and the target site specificity of ISH*27* are identical to those of the *H. volcanii* element family ISH*51* (6). *H. volcanii* contains about 20 to 30 copies of ISH*51*, and members of this insertion element family are highly heterogeneous (6). Comparison of the ISH*27* and ISH*51* variants will help to determine the phylogenetic relationship between insertion elements that occur in different archaebacterial species.

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