Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1017–1020, February 1984
Biochemistry

An immune strain of *Halobacterium halobium* carries the invertible L segment of phage ΦH as a plasmid

(archaebacteria/structural variability/phage variant/insertion element/recombination)

HEINKE SCHNABEL

Max-Planck-Institut für Biochemie, 8033 Martinsried, Federal Republic of Germany

Communicated by Walther Stoeckenius, October 28, 1983

ABSTRACT **The structure of the circular prophage genome of ΦH varies with high frequency in single colony progeny of the defective lysogen *Halobacterium halobium* R_1-3. As in linear ΦH DNA, a segment flanked by two copies of the insertion element ISH1.8 is inverted frequently. This L segment can also circularize to a plasmid of 12 kilobase pairs with simultaneous loss of the remaining phage DNA. Strain R_1-L, which contains this plasmid, is immune to phage infection. A phage variant, ΦHL1, is able to grow on R_1-L and carries an insertion of 1 kilobase pair in its L segment. ΦHL1 does not grow on normal lysogens. This shows that the plasmid confers to R_1-L only part of the immunity of normal lysogens.**

The 59-kilobase-pair (kb) genome of the archaebacterial phage ΦH of *Halobacterium halobium* is terminally redundant and partially circularly permuted. Phage ΦH is a temperate phage. The prophage genome is a covalently closed circle of 57 kb (1).

The DNA of phage ΦH is subject to considerable structural variation. Six phage variants have been described so far that differ by several insertions, a deletion, and an inversion (2). Two of these variants, ΦH2 and ΦH5, both have two copies of the 1.8-kb insertion element ISH1.8 in inverted orientation, whereas the other variants have only one. The frequent inversion of the enclosed DNA segment of 11 kb occurring only in ΦH2 and ΦH5 is apparently correlated to the presence of this inverted repeat.

ISH1.8 appears to be a transposable element not only because of its presence in either one or two copies in the phage genome but also because it occurs in at least two copies in the genome of *H. halobium* (2).

Structural variations in the genome of *H. halobium* have been described first by Pfeifer *et al.* (3) for the 150-kb plasmid pHH1. More recently, several mutations in the bacteriorhodopsin gene have been attributed to insertions (4–7). Sapienza and Doolittle have reported the existence and high variability of families of repetitive DNAs in *H. halobium* (8).

I report here the existence of a ΦH-immune *H. halobium* strain that has lost most of the phage genome and carries only the invertible DNA segment as an independent plasmid of 12 kb. A phage variant, ΦHL1, is able to grow on this strain but unable to infect normal lysogens.

MATERIALS AND METHODS

Bacterial and Phage Strains. All *Halobacterium* strains described here are derived from *H. halobium* R_1, a strain not producing gas vacuoles, which was originally obtained from D. Oesterhelt. *H. halobium* R_1-3 is a defective lysogen that contains the genome of phage ΦH as a plasmid and is immune to phage infection but unable to produce phage (9). The predominant phage variant ΦH1 and the minor variants ΦH2, etc., were isolated by single plaque purification (1, 2). Phage ΦHL1 was obtained by selecting for phage growth on strain *H. halobium* R_1-L but was later grown on strain R_1.

Growth of Bacteria and Phage. The medium contained in 1 liter: NaCl, 250 g; $MgSO_4 \cdot 7H_2O$, 20 g; trisodium citrate·$2H_2O$, 3 g; KCl, 2 g; 50 mM Tris·HCl (pH 7); Oxoid peptone L37 (Basingstoke, England), 15 g. Phage was grown as described (1) but in the above medium. Phage assay, purification of phage, and isolation of phage and cellular DNA followed procedures published earlier (1).

Test of Resistance Against Phage Infection. A late logarithmic or early stationary culture (0.3 ml) was plated in soft agar. A drop of phage solution was placed in the middle, followed by incubation for 3 days. Sensitive bacteria leave a clear zone in the lawn where the drop has been placed; resistant strains show no effect.

Isolation of the Plasmid pΦHL from *H. halobium* R_1-L. Cells grown in 2 liters until late logarithmic phase and harvested by centrifugation were resuspended in salt buffer (3.8 M NaCl/75 mM $MgSO_4$/10 mM trisodium citrate/25 mM KCl/45 mM Tris·HCl, pH 7) up to a total volume of 80 ml. The cells were lysed by dilution with 160 ml of TE buffer (20 mM Tris·HCl/1 mM EDTA, pH 8) followed by a clearing spin for 2 hr at 30,000 rpm in a Beckman type 35 rotor. The plasmid DNA was precipitated from the supernatant with 1 vol of 20% (wt/vol) polyethylene glycol M_r 6000 (in H_2O) for several hours at 4°C. The precipitate was collected by low-speed centrifugation and carefully dissolved in TE buffer up to 30 ml, 28.5 g of CsCl and 0.6 ml of ethidium bromide solution (10 mg/ml) were added, and the plasmids were separated from chromosomal DNA by centrifugation in a Beckman VTi50 rotor. The lower band was collected and recentrifuged to equilibrium in a VTi65 rotor. Ethidium bromide and CsCl were removed by isopropanol extraction and dialysis against TE buffer, yielding a mixture of pΦHL with the 18.5-kb plasmid $pHHR_1$ (9). pΦHL is purified by cleaving $pHHR_1$ with *Eco*RI, which leaves pΦHL in supercoiled form, followed by another CsCl/ethidium bromide centrifugation.

All other methods were described earlier (1).

RESULTS

Inversion and Circularization of a DNA Segment in the Prophage Genome. The DNA of single colony progeny of the defective ΦH-lysogen R_1-3, which carries a circular prophage (9), was analyzed by hybridization with ΦH DNA to look for structural variations like those observed in linear phage DNA. Of the 35 colonies analyzed, 31 DNAs had basically the same structure of the phage genome as R_1-3, illustrated by the hybridization pattern of R_1-3/25 in Fig. 1. Three independent colonies yielded DNA that shows two new fragments of 5.8 and 3.2 kb, typical for the inversion of an 11.5-kb segment of ϕH DNA (Fig. 2), observed earlier in the phage variants ΦH2 and ΦH5 (2). One of the 35 clones termed R_1-L has lost most of the phage genome and shows in the hybridization pattern a new fragment of 4.2 kb. As will

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase pair(s).

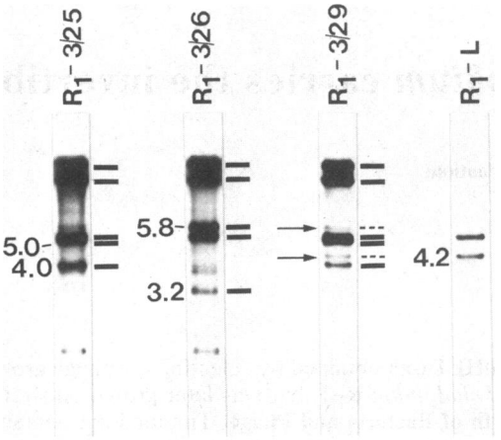

FIG. 1. Autoradiography of the hybridization patterns of ΦH DNA with *Pst* I-digested DNA from progeny of the defective lysogen R_1-3. In most of the clones—e.g., R_1-3/25—the prophage has the same structure as in R_1-3. In R_1-3/26 and two other independent single colonies two restriction fragments are changed, indicating the inversion of a segment of ΦH DNA termed L segment. R_1-L has lost most of ΦH DNA—e.g., the two largest fragments—and the remaining phage DNA is characterized by a new fragment of 4.2 kb. R_1-3/29 shows the basic pattern of pΦH3, but minor bands indicate both the inversion of the L segment and the fragment specific for strain R_1-L. Sizes are indicated in kb.

be shown below, the invertible DNA segment has been circularized (Fig. 2).

A high frequency of both the inversion, indicated by the 5.8-kb *Pst* I fragment, and the circularization, indicated by the 4.2-kb fragment, is illustrated by the presence of both as minor fragments in the DNA of the clone R_1-3/29 (Fig. 1).

Variations in minor hybridizing bands were observed in addition to the major changes visible in Fig. 1 but have not been analyzed so far.

Structure of the Plasmid pΦHL. From *H. halobium* R_1-L, which has lost most of the phage DNA, a plasmid of 12 kb consisting of the remaining phage DNA was isolated. Fig. 3 shows an electron micrograph, and Fig. 4 shows several patterns of restriction fragments of this plasmid, termed pΦHL. A restriction map for *Bam*HI and *Cla* I (Fig. 5) shows that pΦHL consists of that segment of ΦH DNA that can be inverted in ΦH2 and ΦH5, now called the L segment. A region of 800 base pairs, which contains the point of circularization,

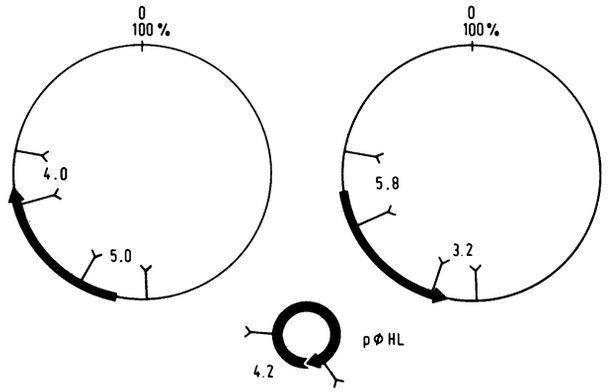

FIG. 2. Schematic representation of the three types of clones derived from the defective lysogen R_1-3. Only those *Pst* I fragments affected by the structural changes are indicated by their sizes (in kb, compare Fig. 1). The structure of the prophage in R_1-3/25 was the same as that in R_1-3. Three clones—e.g. R_1-3/26—are characterized by the inversion of the L segment, and one had only the L segment, circularized to a smaller plasmid.

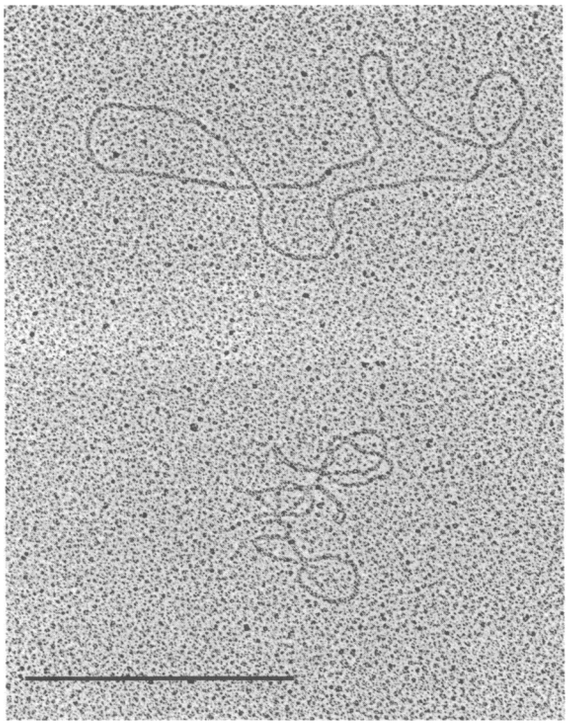

FIG. 3. Electron micrograph of supercoiled and open circular pΦHL. (Bar = 1 μm.)

is too small for a complete copy of the insertion element ISH1.8, which flanks the L segment in phage DNA as an inverted repeat. By further restriction mapping the region containing the point of circularization was reduced to 140 base pairs. There is no ISH1.8 outside of this region, but the presence of a small part of ISH1.8 within these 140 base pairs cannot be exluded.

Properties of Strain R_1-L. As expected, R_1-L does not produce phage because of the deletion of a large part of the phage genome. An even lawn grows across a drop of about 10^5 phages on a plate, and only rarely a few minute plaques are observed. Therefore, R_1-L is immune against phage infection.

A Phage Variant Able to Grow on R_1-L. However, a phage solution of high concentration (10^{12} plaque-forming units/ml

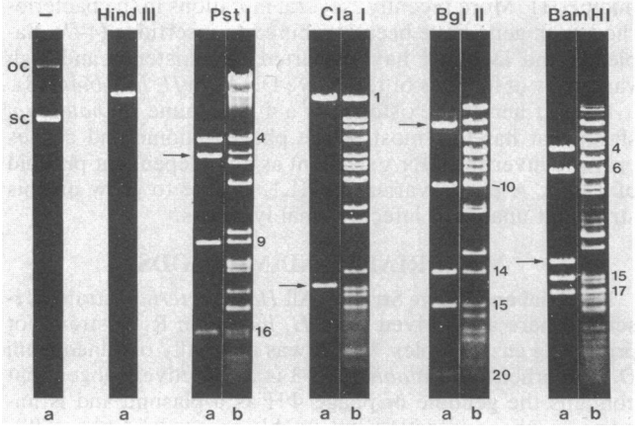

FIG. 4. Restriction patterns of pΦHL in comparison to ΦH1 DNA. *Hin*dIII linearizes pΦHL with a single cut. *Cla* I produces two fragments, one identical to *Cla* I-1 of ΦH1 DNA, the second new in the plasmid. The other restriction enzymes cut pΦHL several times, always yielding one new fragment formed by circularization (arrows). OC, open circular; SC, supercoiled. The numbers of those fragments of ΦH1 DNA that are present in pΦHL are indicated.

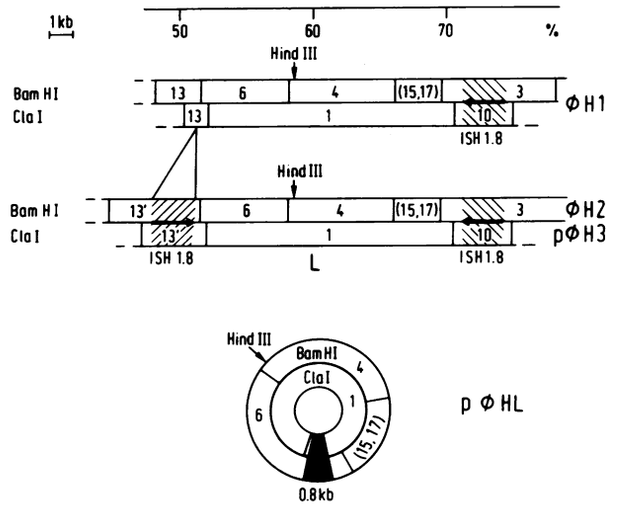

FIG. 5. Restriction map of pΦHL for *Hin*dIII, *Cla* I, and *Bam*HI in comparison to the corresponding region in ΦH1 DNA and in the prophage pΦH3, which contains an additional copy of the insertion element ISH1.8. The region of 0.8 kb containing the point of circularization is shown in black.

on the normal host R_1) does cause growth inhibition of R_1-L on plates and yields a titer of about 10^8 plaque-forming units/ml on R_1-L, but the plaques are extremely small and therefore difficult to see. This indicates that a fraction of 10^{-4} of normal phage consists of variants able to grow on R_1-L. This variant, called ΦHL1, was isolated from plaques on R_1-L. ΦHL1 gives an approximately equal number of plaques on R_1 and on R_1-L, but, in contrast to the small plaques on R_1-L, the plaques on R_1 are of normal size. ΦHL1 does not form plaques on the lysogen R_1-4, which carries the complete phage genome as a plasmid.

Structure of ΦHL1 DNA. DNA isolated from purified phage ΦHL1 was analyzed by cleavage with restriction enzymes (Fig. 6). Comparison with the patterns of ΦH1 DNA shows that ΦHL1 DNA carries an insertion of 1 kb at a position within the L segment, resulting in a size increase of the fragments *Pst* I-4, *Cla* I-1, *Bgl* II-10, and *Bam*HI-4 (Fig. 7). Due to the headful packaging of ΦH DNA, this insertion leads to a shift of the ends of mature DNA molecules by 1 kb

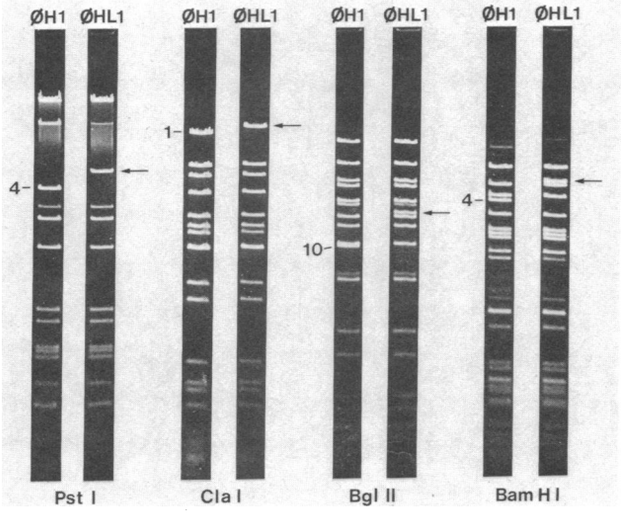

FIG. 6. Restriction patterns of ΦHL1 DNA in comparison to ΦH1 DNA. In each pattern, one fragment of ΦH1 (numbered) has been replaced by a larger fragment in ΦHL1 (arrows). The size difference in each case is 1 kb.

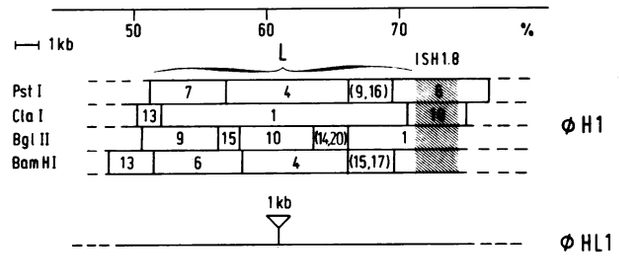

FIG. 7. Structure of ΦHL1 DNA. A restriction map of the region of ΦH1 DNA containing the L segment is shown. The restriction fragments that are larger in ΦHL1 DNA by 1 kb (see Fig. 6) all come from the L segment, placing the point of insertion between map positions 58% and 64%.

relative to those of ΦH1, explaining additional differences between the restriction patterns of ΦH1 and ΦHL1.

DISCUSSION

As shown earlier for linear phage DNA (2), the presence of two copies of the 1.8-kb DNA element ISH1.8 in inverted orientation leads to the frequent inversion of the enclosed L segment. I have now observed that the invertible DNA can also circularize to form a plasmid of 12 kb (pΦHL). This plasmid does not contain a copy of ISH1.8, although the presence of a small part of it cannot be excluded.

The occurrence of three single colonies with the inverted L segment and one with the plasmid pΦHL in a total of only 35 single colonies indicates a high frequency of both inversion and circularization. This is strengthened by the appearance of minor bands specific for the inversion and for the circularization of the L segment in the hybridization pattern of the defective lysogen R_1-3/29. The similarity of the frequencies of the two events may indicate that they involve a similar mechanism of recombination.

One possible mechanism for the inversion of the L segment is homologous recombination at the 1.8-kb inverted repeats. However, this does not explain the circularization of the L segment, which would require the existence of direct repeats. Also, one would expect one copy of ISH1.8 to be present on the circularized DNA—i.e., on pΦHL—which is not the case. Sequence analysis data will have to be obtained before suggesting a possible mechanism for inversion and circularization.

The reciprocal DNA consisting of the prophage with a deleted L segment has never been observed. Together with the loss of all phage DNA except for pΦHL in R_1-L, this indicates that the only origin of replication of ΦH DNA lies on the L segment.

Information about at least one gene present on pΦHL comes from the immunity of R_1-L against phage infection. However, this immunity is only part of the immunity of the other ΦH-lysogens, because the phage variant ΦHL1 cannot overcome the immunity of the strains containing the whole prophage.

The 1-kb insertion in ΦHL1 DNA appears to be responsible for the ability of this variant to grow on R_1-L. The analysis of the two levels of immunity and of the changes caused by the 1-kb insertion may allow a first look into the regulation of gene expression in the archaebacterium *H. halobium*.

I thank Karin Dick for excellent technical assistance and Wolfram Zillig for support, discussions, and reading the manuscript.

1. Schnabel, H., Zillig, W., Pfäffle, M., Schnabel, R., Michel, H. & Delius, H. (1982) *EMBO J.* **1**, 87–92.

2. Schnabel, H., Schramm, E., Schnabel, R. & Zillig, W. (1982) *Mol. Gen. Genet.* **188,** 370–377.
3. Pfeifer, F., Weidinger, G. & Goebel, W. (1981) *J. Bacteriol.* **145,** 375–381.
4. Simsek, M., DasSarma, S., RajBhandary, U. L. & Khorana, H. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7268–7272.
5. Betlach, M., Pfeifer, F., Friedman, J. & Boyer, H. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1416–1420.
6. DasSarma, S., RajBhandary, U. L. & Khorana, H. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2201–2205.
7. Pfeifer, F., Betlach, M., Martienssen, R., Friedman, J. & Boyer, H. W. (1983) *Mol. Gen. Genet.* **191,** 182–188.
8. Sapienza, C. & Doolittle, W. F. (1982) *Nature (London)* **295,** 384–389.
9. Schnabel, H. & Zillig, W. (1984) *Mol. Gen. Genet.*, in press.