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Haloarchaeal virus morphotypes

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Abstract

Hypersaline waters and salt crystals are known to contain high numbers of haloarchaeal cells and their viruses. Both culture-dependent and culture-independent studies indicate that these viruses represent a world-wide distributed reservoir of orphan genes and possibly novel virion morphotypes. To date, 90 viruses have been described for halophilic archaeal hosts, all belonging to the *Halobacteriaceae* family. This number is higher than that described for the members of any other archaeal family, but still very low compared to the viruses of bacteria and eukaryotes. The known haloarchaeal viruses represent icosahedral tailed, icosahedral internal membrane-containing, pleomorphic, and spindle-shaped virion morphotypes. This morphotype distribution is low, especially when compared to the astronomical number ($>10^{31}$) of viruses on Earth. This strongly suggests that only certain protein folds are capable of making a functional virion. Viruses infecting cells belonging to any of the three domains of life are known to share similar major capsid protein folds which can be used to classify viruses into structure-based lineages. The latest observation supporting this proposal comes from the studies of icosahedral tailed haloarchaeal viruses which are the most abundant virus isolates from hypersaline environments. These viruses were shown to have the same major capsid protein fold (HK97-fold) with tailed bacteriophages belonging to the order *Caudovirales* and with eukaryotic herpes viruses. This proposes that these viruses have a common origin dating back to ancient times. Here we summarize the current knowledge of haloarchaeal viruses from the perspective of virus morphotypes.

Keywords

Haloarchaeal virus; virion morphotype; viral lineage; halophilic archaea; hypersaline

1. Introduction

Halophilic (salt-loving) archaea, or shortly haloarchaea, are aerobic, red-pigmented, pleomorphic, coccoid, rod-, or square-shaped cells that require above 1.5 M concentration of NaCl for growth (1). Usually the name haloarchaea refers to archaea in the family *Halobacteriaceae* (phylum *Euryarchaeota*) which at the moment contains around 50 genera of extreme halophiles (2). However, halophilic archaea have also been classified into some genera of methanogens as well as into the newly described class, *Nanohaloarchaea* (2-5). The latter have only been detected by metagenomic analyses, and cultivable isolates are not known (5). Different types of salt lakes and solar salterns are natural environments for haloarchaea. The waters are often colored red due to the presence of haloarchaeal carotenoid pigments. Hypersaline environments reside mostly in the tropical and subtropical areas, but can be encountered even in the Antarctica, as high salinity prevents the formation of ice at temperatures as low as -20 °C (6, 7). Haloarchaea have been isolated from all these environments worldwide. In addition, these microorganisms are also found from crystallized, even food-grade, salt as well as from ancient salt deposits (8-12).

Like many extremophiles, haloarchaea lack natural predators (13). Although some single celled flagellates and ciliates are known to feed on these archaea (14), it is considered that the major evolutionary pressure may be caused by viruses (15, 16). Hypersaline environments are known to contain high numbers of prokaryotic viruses and because archaea dominate in these environments, their viruses are considered the most abundant. Haloarchaeal viruses can outnumber their host cells up to hundred fold (15, 17, 18). Currently, 90 haloarchaeal viruses have been described and they all infect archaea belonging to the family *Halobacteriaceae* (Tables 1-4) (19-21). The hosts of these viruses belong to the

genera *Halobacterium*, *Haloarcula*, *Halorubrum*, *Haloferax*, *Haloterrigena*, *Halogramum*, *Halobellus*, *Halosarcina*, *Halogeometricum*, *Natrinema*, and *Natrialba* (19-21). All the known archaeal viruses have DNA genomes (20). Most haloarchaeal virus genomes consist of dsDNA, but viruses with ssDNA genomes are also known. Persistent infection seems to be characteristic for many haloarchaeal viruses, although different temperate and virulent viruses have also been described (19, 22).

1.1 Haloarchaeal virus isolation

The history of archaeal virus isolation is short (Fig. 1). The first virus infecting an archaeal host was actually the halophilic tailed icosahedral virus Hs1, which was isolated and described during the 1970's before archaea were established as a distinct domain of life (23, 24). From then on until 1998, all the isolated haloarchaeal viruses represented tailed icosahedral morphotypes with either contractile or non-contractile tails (25, 26). In 1998, the first spindle-shaped haloarchaeal virus, His1, was described followed by His2 which was later reclassified as a pleomorphic virus (27-29). In 2005, the first icosahedral haloarchaeal virus, SH1, was characterized (30). At that time, the total number of haloarchaeal viruses was still around 15. From 2010 onwards, two large-scale global expeditions, one spatial and another temporal, were performed drastically increasing the number of known haloarchaeal virus isolates (Fig. 1a-c, Fig. 2a-f) (9, 21). The dominance of tailed icosahedral morphotypes was still evident and the number of spindle-shaped viruses was not increased (Fig. 1b; Tables 1-4). However, a distinct group of archaeal viruses with a novel, pleomorphic morphotype was recognized (Fig. 1a-c, Fig 2f; Chapter 5; (29, 31)). HRPV-1 was the first described archaeal virus with an ssDNA genome (31). The number of icosahedral internal membrane-containing virus isolates was increased with two new viruses and during recent years, two more were

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4 isolated from different parts of the world (9, 21, 32, 33). Despite their distant isolation
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6 locations, these viruses are closely related (Chapter 3). The spatial and temporal studies
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8 revealed that actually all haloarchaeal viruses are part of a global network with hundreds of
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10 virus-host interactions occurring over time and space (9, 21).
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15 **1.2 Haloarchaeal virus morphotypes and viral lineages**

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18 Among the known haloarchaeal virus isolates, tailed icosahedral viruses represent the most
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20 dominant group, but pleomorphic, icosahedral internal membrane-containing, and spindle-
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22 shaped viruses have also been described (19, 34). Interestingly, to date archaeal tailed
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24 icosahedral viruses have only been described for euryarchaeal hosts, although such virus
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26 morphotypes have been recognized by transmission electron microscopy (TEM) in
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28 hyperthermic environments that are dominated by crenarchaea (35). The spindle- or lemon
29
30 shape is considered specific for archaeal viruses and it is the most frequently observed
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32 morphotype by TEM studies of hypersaline environmental water samples (15, 18, 36).
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34 Although many spindle-shaped viruses have been isolated from hyperthermic environments,
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36 currently His1 remains the only known haloarchaeal virus isolate with this morphology (Table
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38 4) (See Chapter 4).
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45 It has been suggested that viruses can be grouped into structure-based lineages based on
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47 conserved architectural elements such as the major capsid protein (MCP) fold and virion
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49 assembly principles (37). Viruses belonging to a lineage are suggested to have a common
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51 ancestor (37). To date, four viral lineages (PRD1-like, HK97-like, blue tongue virus-like, and
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53 picorna-like) based on similar MCP folds have been established and many more are thought
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55 to exist, suggesting that viruses have a polyphyletic origin (37, 38). Two of the established
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57 lineages comprise viruses infecting organisms from all the three domains of life. These are
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the lineage of PRD1-like viruses for icosahedral internal membrane-containing viruses and the lineage of HK97-like viruses for tailed icosahedral viruses and herpes viruses (38). Currently only the crenarchaeal virus STIV has been shown to belong to the PRD1-lineage, because high resolution structures of the MCPs are not available for other archaeal viruses representing the icosahedral internal membrane-containing morphotype (39, 40). Moreover, this lineage is considered to have two subdivisions. One of them contains viruses with the characteristic double beta barrel MCP fold exemplified by the type virus, bacteriophage PRD1, and the crenarchaeal STIV (39, 41). The second subdivision contains viruses with two MCPs such as the haloarchaeal icosahedral internal membrane-containing viruses (Chapter 3).

The characteristic HK97 fold was recently identified in the archaeal tailed icosahedral virus HSTV-1 indicating that archaeal tailed icosahedral viruses have common ancestry with eukaryotic herpes viruses and tailed bacteriophages (Chapter 2.2) (42). In addition to these well-established lineages, the short-tailed spindle-shaped crenarchaeal and euryarchaeal viruses are suggested to belong to a common lineage (Chapter 4) (43), and the pleomorphic haloarchaeal viruses might share a lineage with the bacterial mycoplasma viruses (Chapter 5). However, MCPs of these viruses have not yet been identified.

The observed diversity of different virion morphotypes infecting archaea (especially crenarchaea) compared to the low architectural diversity of bacteriophages (~96 % tailed icosahedral phages) has raised interesting questions. It has been speculated, that the more primitive and simple archaeal cell wall could be one reason for the greater virus diversity as bacterial cell walls are complex and more difficult to penetrate (44). Bacteria have cell walls consisting of peptidoglycan and often an outer membrane while archaea lack peptidoglycan and most often have a proteinaceous S-layer (45). The coccoid haloarchaea on the other

hand, have more rigid cell envelopes (1) but to date, no information about their viruses is known. Here, we present the known haloarchaeal viruses based on their specific morphotypes.

2. Tailed icosahedral viruses

Haloarchaea are infected by both virulent and temperate tailed icosahedral viruses representing all the three morphotypes established for tailed bacteriophages (*Caudovirales*) (34). Interestingly, the majority of the described archaeal tailed icosahedral viruses are myoviruses while siphoviruses dominate bacteriophage isolations (Tables 1-2) (34).

After the isolation of Hs1, several more tailed icosahedral haloarchaeal viruses with either contractile or non-contractile tails were described. Most (if not all) of these viruses infected different strains of *Halobacterium salinarum* (25). Some biology and genome information is known for these viruses, but detailed structural studies and genomics were missing until very recent times. Nowadays, three dimensional structures of three haloarchaeal tailed icosahedral viruses have been resolved (myo-, sipo-, and podoviruses) (42, 46) and the total number of described haloarchaeal tailed icosahedral viruses is 73 (Tables 1-2).

To date, altogether 17 haloarchaeal tailed icosahedral virus genomes have been completely sequenced ranging from 36 kb (HRTV-4) to 144 kb (HGTV-1) (47). Genomes of the described archaeal tailed icosahedral viruses consist of linear dsDNA and resemble the genomes of tailed bacteriophages by gene organization (47-49). Comparative bioinformatic analyses of the genomes of archaeal and bacterial tailed icosahedral viruses suggest that these viruses have common structural and assembly features (48). The function of a high number of predicted genes of the tailed archaeal viruses have remained unknown and very few of these

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4 genomes have been found to be closely related to each other (50). Due to the high number of
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6 described tailed icosahedral viruses infecting haloarchaea, only the most interesting or well-
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8 characterized ones are introduced in the following text. The names, morphotypes, original
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10 host organisms, and genome sizes (when available) of all the described haloarchaeal tailed
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12 icosahedral viruses are listed in Tables 1 and 2.
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16 17 18 **2.1 Tailed icosahedral viruses with contractile tails** 19 20

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22 The first described archaeal virus, Hs1, has an icosahedral 50 nm head and a 120 nm long
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24 contractile tail. No information about the genome of this virus is available. Hs1 was isolated
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26 from flagellar preparations of *Halobacterium salinarium* strain 1 (23). The virus requires high
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28 NaCl concentrations for infectivity and is able to establish a carrier state with the host in
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30 salinities between 3.5 M and 4.0 M NaCl (23, 51). Release of Hs1 progeny is known to last
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32 over 30 hours and is lengthened at high salinities. The adsorption rate of the virus to its host
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34 is higher at lower NaCl concentrations in which the virus enters lytic growth instead of carrier
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36 state (25). In the carrier state, infected cells of *Hbt. salinarium* can survive several
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38 generations without releasing viruses. Apart from true lysogeny, Hs1 carrier state is unstable
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40 described by sudden bursts of numerous Hs1 particles from the infected cells (51).
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47 Virus S5100 infecting *Halobacterium cutirubrum* is another virus for which the effect of salinity
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49 has been studied in more detail (52). The virulent myovirus was originally isolated from a
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51 Jamaican salt pool. While around 4 M NaCl concentrations are optimum for S5100, 3 M and
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53 close to saturated concentrations of NaCl (5.2 M) induce persistent infection in which large
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55 fraction of the host populations survives, although containing the virus. This effect is
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57 reversible, because when the virus is introduced back to lower NaCl concentrations (3 M or
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4 3.5 M) it continues to infect cells in a virulent fashion. The burst size and latent period of the
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6 infection correlate negatively with increasing salinity. The plating efficiencies of the virus are
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8 reduced at salinities lower or higher than the optimum (52). The authors suggested that
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10 haloarchaeal viruses might refuge the cells when salinities become less optimal for cell
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12 survival.
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16 During 1970's and 1980's several tailed icosahedral viruses were isolated from spontaneous
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18 lysates of *Hbt. salinarum* (25). One of these is the temperate myovirus Φ H (53). The virus has
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20 a 64 nm head size, a 170 nm long contractile tail, and the genome is 59 kb linear dsDNA,
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22 which is circularly permuted and terminally redundant due to a headful packaging mechanism
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24 (53). In provirus state, Φ H genome can circularize into a plasmid if it contains two copies of
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26 an insertion element called ISH1.8 (54). This genomic region is called the L-segment, and it
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28 gives the host partial immunity against the virus (54). Φ H does not integrate into the host
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30 genome, but replicates as a plasmid in the host cytoplasm. ISH1.8 is also present in the host
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32 genome. The host is genetically very unstable and spontaneous release of several different
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34 genetic variants of Φ H is common (25). Φ H variants differ from each other by having
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36 deletions, insertions, or inversions in the genome. Many of these variant types are genetically
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38 very unstable resulting in the accumulation of more and more variants during plaque
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40 purification (25). Otherwise the genome size and titer on host are the same as for Φ H. All the
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42 known variants contain one or two copies of the insertion element ISH1.8 and no viruses
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44 without the element are known (54). In addition to lysogeny, Φ H can establish a carrier state
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46 with the host. The carrier state was suggested after observation of host cells that contained
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48 the virus, but were still sensitive to superinfection (lysogens of Φ H are resistant to infection)
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50 (25). Φ H requires above 3 M concentrations of NaCl or KCl, but can tolerate lower
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52 concentrations in the presence of 0.1 M Mg^{2+} (25).
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4 Φ Ch1 is the second described haloarchaeal temperate tailed icosahedral virus and one of the
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6 best studied haloarchaeal tailed viruses (55, 56). Although Φ Ch1 infects a haloalkaliphilic
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8 host, *Natrialba magadii*, and thus has been isolated from a very different environment
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10 (alkaline soda lake) than the other haloarchaeal viruses, the MCP encoding genes of Φ Ch1
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12 and Φ H share 80 % sequence similarity (57). The genome of Φ Ch1 is 58.5 kbp linear dsDNA
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14 (55). Unlike Φ H, Φ Ch1 integrates into the host chromosome (55). An interesting feature of
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16 Φ Ch1 is that it is known to package both host derived and viral RNA into its virion (56). The
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18 virus is known to produce two types of tail fibers of which only one type is able to bind to its
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20 host (58). It is considered that galactose residues are related to cell adhesion since
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22 galactosidase treatment of the virus was found to inhibit attachment (58).
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28 The virulent haloarchaeal myoviruses HF1 and HF2 were isolated from the same Australian
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30 saltern pond (59). Genome sizes of the viruses are 75.9 kb and 79.7 kb dsDNA, respectively,
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32 and with 94.4 % identity to each other, these viruses are an example of the high level of
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34 recombination in hypersaline environments (60). The host ranges of the two viruses are very
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36 different. HF1 infects haloarchaea belonging to the genera *Haloterrigena*, *Haloferax*,
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38 *Haloarcula*, and *Halobacterium*, while HF2 is specific to *Halobacterium saccharovorum* (59,
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42 60). Recently, two more tailed icosahedral viruses, HRTV-5 and HRTV-8 with contractile tails
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44 related to HF1 and HF2 have been described (50). All these viruses originate from different
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46 environments, but most of their encoded proteins are homologs. The most conserved proteins
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48 within this virus group are those related to virion structure and assembly (47).
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53 HSTV-2 is currently the only halophilic myovirus which has been studied in structural detail.
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55 Cryo-electron microscopy (Cryo-EM) based icosahedral structure of HSTV-2 revealed that the
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57 capsid has T=7 symmetry (46). It has been suggested that HSTV-2 uses a stabilizing protein
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59 to enlarge its capsid since the virus genome size (68.2 kb) is the largest known for T=7
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viruses (46). This represents a unique mechanism not encountered before for any virus (46). HSTV-2 is infective at high and moderate salt concentrations, but loses infectivity in the absence of NaCl. The infectivity, however, is regained when the viruses are transferred back to at least 1.2 M NaCl (46). No morphological changes or genome release was detected in the absence of salt. HSTV-2 genome is related to the genomes of HF1, HF2, HRTV-5, and HRTV-8 (47, 50).

2.2 Tailed icosahedral viruses with non-contractile tails

Haloarchaeal viruses with non-contractile tails are generally less studied than those with contractile tails. These morphotypes resemble the classical siphoviruses and podoviruses of *Caudovirales*. Φ N, Hh1, Hh3, and S45 are among the earliest described viruses with non-contractile tails (Table 2) (25). Φ N differs from most haloarchaeal viruses, because it can tolerate low salinities or the complete absence of salt (NaCl or other salts) (25, 61). The fully cytosine methylated genome of Φ N is a 56 kbp linear dsDNA molecule which is terminally redundant and circularly permuted (61).

Unlike most of the known haloarchaeal viruses which are usually isolated from natural hypersaline environments, siphoviruses Hh1 and Hh3 were isolated from fermented anchovy sauce reflecting the wide distribution of haloarchaeal viruses (62). Hh1 and Hh3 have genome sizes of 37.2 kb and 29.4 kb, respectively. The head diameter of Hh1 is 60 nm, and tail length is 100 nm. The capsid diameter and tail length ratio of Hh3 are different from Hh1. Capsid diameter of Hh3 is 75 nm and tail length only 50 nm. Contrary to Φ N, these viruses do not survive at low or even intermediate salinities (62). Hh3 requires up to 4 M NaCl for infectivity whereas Hh1 is stable at 4.3 M NaCl, but can withstand lower concentrations if sufficient

amount of magnesium is available (0.08 M MgSO₄). Both viruses seemingly establish carrier state with the host (62).

S45 and Ja1 are haloarchaeal siphoviruses which have been isolated from Jamaican salterns. S45 is a virulent virus with remarkably large burst size (1300 viruses are released per cell) (63). Ja1 is one of the earliest described archaeal viruses as it was isolated in 1975 (64). The virus which infects *Halobacterium cutirubrum* and *Halobacterium halobium*, has a 90 nm icosahedral head and a 150 nm long tail (64). Ja1 is extremely sensitive to desiccation (64).

BJ1 is a siphovirus isolated from the hypersaline Lake Bagaejinnor in Inner Mongolia, China (65). The virus, which infects a *Halorubrum* isolate from the same environment, has a capsid diameter of 56 nm and a 71 nm long tail. The genome is a 42.3 kb linear dsDNA molecule which was observed to be terminally redundant (65). The genome has low identity to the previously described haloarchaeal viruses, although some hits to metagenomic sequences related to BJ1 genome have been detected from hypersaline environments in Australia (66).

HVTV-1 is currently the only siphovirus which has been characterized in structural detail (46). HVTV-1 MCP capsomers follow the icosahedral T=13 symmetry. In addition, fivefold symmetric spikes at the vertices and trimeric decorative structures in the centers of the capsomers were observed. The HVTV-1 genome is a 101.7 kb long dsDNA molecule related to siphoviruses HCTV-1 and HCTV-5 infecting *Haloarcula californiae*. Otherwise no close overall similarity to other known virus genomes has been detected (46). However, a few significant matches to bacteriophage head and tail genes as well as genes for nucleotide metabolism and DNA replication were identified. Interestingly, a match to a bacterial and archaeal toxin/antitoxin system was reported, although the function of this HVTV-1 gene

product homolog remains unknown (46). Like the myovirus HSTV-2, HSTV-1 is also reversibly inactivated by low salinity (46).

Podoviruses are characterized by an icosahedral head and a non-contractile short tail that resides partly inside the capsid. This structure is exemplified by bacteriophage T7 (67). It is considered that podoviruses are most common in marine environments, but more rarely isolated due to their narrow host ranges (68, 69). The only known archaeal podovirus is HSTV-1 infecting the strain "Haloarcula sinaiensis" (42). This virus was isolated from an Italian saltern in Margherita di Savoia (9). HSTV-1 has an isometric head of 50 nm and a short ~30 nm non-contractile tail as well as at least five tail fibers attached to the base plate (42). The virion is surrounded by cone-shaped towers that possibly help stabilize the capsid architecture which follows T=7 symmetry (Fig. 3a-b). Cryo-EM based structure at 8.9 Å revealed that the major structural protein gp14 of HSTV-1 exhibits the same HK97-fold known for tailed bacteriophages and eukaryotic herpes viruses (Fig. 3c-d) (42). The virus can tolerate low NaCl concentrations (1.2 M), but loses infectivity in the absence of NaCl. This can, however, be restored by transferring the virus back to high salinity (above 1.2 M NaCl) (42). The proteins encoding structural genes of HSTV-1 are organized in the same fashion as for tailed bacteriophages. Otherwise, most of them have unknown functions or no matches to public databases. A headful DNA packaging mechanism was suggested due to circular permutation of the genome as well as recognition of the potential portal and terminase genes (42).

3. Icosahedral inner membrane-containing viruses

Organisms in all the three domains of life are infected by icosahedral viruses with a characteristic lipid membrane inside the capsid (Fig. 3e). To date, five haloarchaeal internal

membrane-containing viruses, SH1, PH1, HHIV-2, SNJ1, and HCIV-1, infecting haloarchaea belonging to the genera *Haloarcula*, *Halorubrum*, or *Natrinema*, are known (Table 3) (30, 32, 33, 40, 70). SH1, PH1, and HHIV-2 all infect *Haloarcula hispanica* and are closely related with high genome sequence similarity (approximately 60 % between SH1 and HHIV-2 and above 70 % between SH1 and PH1). These viruses have linear dsDNA genomes and virulent life cycles. SNJ1 infecting a strain of *Natrinema*, is more distantly related to the other viruses, has a circular dsDNA genome, and a temperate life style (33). HCIV-1 was recently isolated and no detailed information is yet known about this virus (21).

SH1 was the first icosahedral internal membrane-containing virus described for halophilic archaea. The virus was isolated from an Australian hypersaline Serpentine Lake (30). Currently, SH1 is the only representative of the haloarchaeal icosahedral internal membrane-containing viruses for which the structure has been resolved by cryo-EM and three-dimensional (3D) reconstruction (Fig. 3e-f) (71). SH1 virion diameter is 70 nm and the genome size is 30.9 kb linear dsDNA (72). In addition to its isolation host, SH1 also infects a strain of *Halorubrum* isolated from an Australian salt lake (30). SH1 requires above 1.5 M NaCl and/or 2 M MgCl₂ for virion stability (30). The lipid composition of the viral membrane (Fig. 3e) consists of phosphatidylglycerol (PG), phosphatidylglycerophosphate methyl ester (PG-Me), and phosphatidylglycerosulphate (PGS). (72). In addition, 12 % of the viral lipids consist of neutral lipids. The lipids are present in the virus in different proportions compared to the host membrane (72). SH1 virion is composed of 11 identified structural protein species either forming the capsid or interacting with the membrane. A putative packaging ATPase similar to internal membrane-containing bacteriophages was identified being the only ORF from the 56 predicted ones (ORF 17) with sequence similarity matches in the database at the time of the study (72). From the confirmed virion proteins, VP3, VP4, and VP7 are the most

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4 abundant ones and the proteins associated with virion coat. VP12 has two membrane-
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6 spanning helices and is the major structural membrane protein (72, 73). VP4 and VP7 are the
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8 MCPs that form the viral capsid which is arranged according to T=28 dextro lattice (71). VP3
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10 and VP6 form the horn-like virion spikes at the five-fold vertices (Fig. 3f) (71).

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14 PH1 was isolated from the Australian Pink Lake which is located next to Serpentine Lake from
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16 where SH1 was found. The genome is 28.1 kb linear dsDNA with high nucleotide identity to
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18 SH1 (74 %). However, PH1 contains some divergent genomic regions and the virion diameter
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20 is reported to be 51 nm. PH1 has highly similar protein profile, and the same number of
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22 structural protein genes as SH1 (32). Also the host ranges of the two viruses are similar. The
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24 infection curve of PH1 also resembles that of SH1, but fewer cells are infected. Viral DNA of
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26 PH1 is able to transfect six species of haloarchaea belonging to the genera *Haloarcula*,
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28 *Haloferrax*, *Halorubrum*, *Haloterrigena*, and *Natrialba* (32).

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33 HHIV-2 was the second described haloarchaeal internal membrane-containing virus and is
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35 related to SH1 despite being isolated from a different continent (an Italian saltern of
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37 Margherita di Savoia) (9, 40). HHIV-2 has a capsid diameter of around 80 nm and the
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39 genome is a 30.6 kb long linear dsDNA molecule (40). HHIV-2 life cycle is virulent and the
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41 burst size is approximately 180 per cell. In addition to its isolation host, HHIV-2 also infects
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43 *Haloarcula* sp. PV7 isolated from Trapani, Italy. HHIV-2 adsorption is slightly faster than that
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45 of SH1. Unlike SH1, HHIV-2 can withstand low NaCl concentrations (0.1 M) and the titer is
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47 reduced 100-fold in the total absence of salt (40). Calcium or magnesium does not have an
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49 effect on the virus stability. The genome of HHIV-2 shares ~59 % similarity to the genome of
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51 SH1, the organization of the genes is similar for the two viruses, and the HHIV-2 structural
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53 protein profile resembles that of SH1 with minor differences (40). From the 43 putative ORFs
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55 of HHIV-2, 32 are related to SH1. The highest amino acid identities were detected for the two
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4 MPCs, VP4 and VP7, as well as the putative ATPase, and the membrane protein VP12. The
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6 putative ATPase gene of HHIV-2 resembles those found in the other icosahedral internal
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8 membrane-containing viruses. The genes encoding for the spike protein complex (putative
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10 spike proteins for HHIV-2) are different between SH1 and HHIV-2 (40). This is, however,
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12 expectable since viral elements related to host cell recognition have been observed to
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14 undergo rapid evolution (37, 74). Due to the high resemblance of the MCPs of SH1 and
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16 HHIV-2, it is suggested that also HHIV-2 belongs to the PRD1-like viral lineage subgroup
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18 which contains icosahedral internal membrane-containing viruses with two MCPs (See
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20 Chapter 1.2) (40). Both SH1 and HHIV-2 are also related to certain plasmids identified in the
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22 haloarchaeal genomes (40, 75).
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28 SNJ1 is the first and currently the only known temperate haloarchaeal icosahedral
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30 membrane-containing virus. The virus was isolated from a lysogenic strain of *Natrinema* sp.
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32 J7-1 by mitomycin C induction and it plates on *Natrinema* sp. J7-2 (33). Both strains have
33
34 been isolated from a Chinese salt mine (76). SNJ1 has a genome size of 16.3 kbp and apart
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36 from the other known haloarchaeal icosahedral viruses with a membrane the genome is a
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38 circular dsDNA molecule. The genome is identical to a circular plasmid pHH205 which has
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40 been identified in the host genome indicating that SNJ1 can have a provirus state. The
41
42 presence of a lipid membrane and the comparison of the putative ATPase sequence indicate
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44 that the virus belongs to the lineage of PRD1-like viruses. When first recognized, SNJ1
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46 infected J7-2 with titers as low as 10^4 pfu/ml. Mitomycin C and additional optimization
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48 techniques (33) were able to enhance the virus yield up to 10^{11} pfu/ml. Burst size is around
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50 100 pfu/cell. With the most optimal infection conditions, approximately 95 % of the cells
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52 become infected. As for the other icosahedral membrane-containing viruses, SNJ1 acquires
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54 its lipids selectively from the host. The data on SNJ1 MCPs is very preliminary, but suggests
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that the virus has two MCPs, designated as PB2 and PB6 (33). The virus has two ORFs similar to those of SH1. Otherwise, the genome is quite distinct from the other halophilic icosahedral inner membrane-containing viruses.

4. Spindle-shaped virus His1

In addition to hypersaline environments, spindle-shaped viruses are also common in hyperthermic environments, as well as in acidic mines and even in some fresh water environments (35, 77-79). Most of the currently known spindle-shaped viruses infect crenarchaeal hosts (43). In addition, a few spindle-shaped virus-like particles infecting euryarchaeal deep-sea anaerobes from the genera *Pyrococcus* or *Thermococcus* as well as methanogens in the genus *Methanococcus* are known (20). However, so far, only one spindle-shaped haloarchaeal virus, His1, has been described (27).

His1 was isolated from the saltern of Avalon, Victoria, Australia (27). Host range is restricted to the isolation host *Hal. hispanica* and the infection is persistent without lysis of the host cells (29, 80). Recent cryo-EM and 3D reconstruction studies have revealed that the flexible His1 spindle-shaped virion is approximately 90 nm in length and 45 nm in width and the particles are somewhat pleomorphic lacking strict symmetry (81). The tapering end of the virion includes a short tail with six tail fibers (81). His1 virions can transform into long tubular structures. This transformation is considered to be related to events associated with the virus entry (81). The 3D structure of the crenarchaeal virus SSV1 was defined at the same time than that of His1 revealing that these two spindle-shaped viruses of very different origins are structurally similar (82). Like His1, SSV1 also has a short tail with six tail fibers and it has been suggested that these fibers could be used for attachment to the host cell surface (82).

Apart from the crenarchaeal spindle-shaped viruses with circular dsDNA genomes, His1 genome is linear dsDNA of 14,464 bp (28) and likely contains terminal proteins at both ends of the genome (28, 83). His1 is one of the few haloviruses encoding its own polymerase which is a putative type B DNA polymerase suggesting protein primed replication (28). ORF 16 is predicted to code for a putative ATPase (80). His1 can tolerate a wide range of salinities and remains infective even at 100 mM NaCl. The purified virion protein profile consists of two major structural proteins (approximately 8 and 11 kDa) that are encoded by the same gene. The 11 kDa protein was confirmed to be a lipid-modified version of the smaller protein. Otherwise no membrane was detected in the virion (80).

To date, His1 remains the only haloarchaeal virus for which DNA ejection has been studied by single-molecule techniques (83). The ejection process was found to be unidirectional and randomly paused. Osmotic pressure as well as increased Mg^{2+} concentration had an inhibitory effect on DNA ejection. The mean ejection velocity was around $50 \mu m s^{-1}$ or $150 kbp s^{-1}$, but varies as a function of ejected genome length, slowing down towards the end. This type of velocity is comparable to that of tailed icosahedral bacteriophages (83). The incompleteness of the DNA ejection suggests that cellular processes are required in the process.

His1 has been classified in the floating genus *Salterprovirus* without a specific viral family (28). Recent comparative bioinformatic study revealed that the MCPs of all the spindle-shaped viruses with short tails infecting crenarchaeal or euryarchaeal hyperthermophiles, or euryarchaeal halophiles, share above 50 % pairwise sequence similarity and the presence of two similar hydrophobic domains (43). In addition, putative AAA+ type ATPases have been identified for all these viruses. Consequently, it was proposed that all the short-tailed spindle-shaped viruses should be classified in the family *Fuselloviridae* and His1 would be the only

representative of the newly proposed genus *Epsilonfusellovirus*. Such similarities in the MCPs and ATPases of different viruses suggest evolutionary relatedness (43).

5. Pleomorphic viruses

Archaeal viruses with a pleomorphic virion architecture have currently only been described for haloarchaea from the genera *Halorubrum*, *Haloarcula*, or *Halogeometricum* (Table 5). From the 11 known haloarchaeal pleomorphic viruses HRPV-1 is the best studied and the type virus which was also the first known archaeal virus with an ssDNA genome (7048 nt circular ssDNA) (31). Later on several other viruses similar to HRPV-1 were isolated. Although these viruses had different types of DNA genomes (Table 4), they are related to each other by genome organization and nucleotide identity and have been classified in the proposed viral family “Pleolipoviridae” (29, 84, 85). The most recently isolated pleomorphic viruses HRPV-7, HRPV-8, and HAPV-2 lack further characterization (21).

The virions of pleomorphic viruses are membrane vesicles decorated by randomly distributed spikes (Fig. 1; Fig. 2a). Virion diameters range between 40 nm and 70 nm with HRPV-1 having the smallest and His2 the largest particle size (29). Viral DNA is located inside the membrane vesicle and is free from nucleoproteins (86). The structural protein profile of these viruses consists of two major structural protein species. The spike protein protruding from the membrane surface is around 60-70 kDa and the internal membrane protein size ranges from below 10 to approximately 15 kDa (29). Interestingly, His2 has two spike protein species and HGPV-1 two membrane proteins. In addition, the spike proteins of these two viruses have lipid modifications (29) whereas the spike protein of HRPV-1 is glycosylated (86). It is considered that interactions between the internal membrane protein(s) and the genome drive the virion assembly of pleomorphic viruses (29).

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4 The plaques produced by pleomorphic viruses are hazy and turbid indicating non-lytic virus
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6 release from the cells. Life cycles have been studied in detail for the eight characterized
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8 pleomorphic viruses which all were observed to retard host growth without lysis (29, 31, 85,
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10 87). HRPV-6 and His2 produce the highest number of viruses and cause the most significant
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12 host growth retardation. HGPV-1 infection does not seem to affect host growth rate, and the
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14 viable cell numbers at the stationary growth phase are similar in the infected and non-infected
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16 cultures. The other viruses fall somewhere between these two types (29). Based on these
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18 observations, budding seems the most probable exit mechanism for the haloarchaeal
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20 pleomorphic viruses.
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26 Viral lipids of HRPV-1 have been identified as PG, PG-Me and PGS as well as cardiolipin and
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28 the lipid acquisition from the host is non-selective (86). HRPV-2, HRPV-3, HRPV-6, HHPV-1,
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30 and His2 contain the same lipids as HRPV-1 while HGPV-1 lacks PGS. All these viruses
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32 acquire their lipids non-selectively from the host, although minor differences have been
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34 observed (29). Lipids of HHPV-2 as well as the newly described pleomorphic isolates are not
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36 known.
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40 The host range of pleomorphic viruses is narrow and all viruses infect only their isolation
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42 hosts. However, for HHPV-1 and HHPV-2 as well as for His2, the host has been isolated from
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44 a different country than the virus (Table 4) (85, 87). Interestingly, a CRISPR-spacer sequence
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46 that partially matches the viral DNA of HHPV-2 was identified in the genome of *Har. hispanica*
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48 (85). Altogether the described pleomorphic viruses have been isolated from six different
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50 countries on three continents (Italy, Thailand, Israel, China, Australia, and Spain) indicating
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52 world-wide distribution (29, 85).
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57 Although the described pleomorphic viruses are very similar, their genome types can be
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59 either circular or linear dsDNA or circular ssDNA. These viruses share a set of four to five
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genes including those encoding for the spike, membrane, and putative ATPase proteins (84, 85). Only His2 encodes a type B DNA polymerase (28). Viruses HRPV-3 and HGPV-1 were found to contain unusual single-stranded regions in their genomes. A DNA motif “GCCCA” preceding these single-stranded regions was recognized in HRPV-3 and a similar one is suggested to be found from the genome of HGPV-1 too (84).

The conserved “core” genes have been identified also in the putative proviral elements in the genomes of *Haloferax volcanii* DS2, *Halomicrobium mukohataei* DSM 12286, and in plasmid pHK2 of *Haloferax lucentense* (84, 86, 87). These proviral elements contain genes for integrases which have not been identified in any pleomorphic virus genome (84). The relatedness and possible evolutionary connection of haloarchaeal pleomorphic viruses, proviruses, plasmids, and membrane vesicles produced by archaea, has been discussed (88, 89). In addition, these viruses might be structurally related to the pleomorphic bacteriophages L2 and L172 infecting mycoplasmas (90).

6. Culture-independent halovirus studies

The sequences of the described haloarchaeal virus isolates are rarely detected in the metaviromes of the studied hypersaline environments (66, 91, 92). This, however, is quite evident taken in account the low amount of available sequence information from haloarchaeal viruses (Tables 1-4). Although archaea dominate in these environments, different halophilic bacterial groups are frequently encountered and even some eukaryotes withstand high salinities (5, 13). Thus, these metaviromes most likely consist of sequences derived from viruses infecting hosts from all three domains of life. In addition, virus-like elements such as plasmids or cryptic proviruses can also be abundant in the environment (93).

All the particle morphotypes represented by the isolated viruses (Figs. 1-2) have also been detected by TEM in hypersaline environments (18, 36, 94). However, tailed icosahedral viruses have been reported to contribute less than 5 % of the virus-like particles (VLPs), spindle-shaped VLPs over 40 %, and tailless icosahedral or spherical particles below 30 % (18). In addition, linear particles as well as particles with different types of morphologies previously not described for viruses have been reported (18, 36). While the tailed icosahedral *Caudovirales* morphology most probably refers to genuine viruses, the other morphotypes are less reliable in this sense. For instance, negative stains used in TEM are known to greatly influence particle morphology and infectivity of pleomorphic viruses and probably other viruses too (29). The demand for high NaCl concentration for infectivity is characteristic for most of the described haloarchaeal viruses, but a challenge for electron microscopy. In addition, as mentioned before, it is hard to distinguish between viral and non-viral membrane vesicles.

Studies on virus host dynamics suggest that microorganisms and their viruses are dynamic on the strain or genotype level, but species diversity remains stable over time and space (16). Microbial communities in hypersaline environments are considered to be very specific and harbor previously uncharacterized genetic material (66). However, many similarities have been detected between viromes of geographically distant environments (92, 95). For example, nucleotide sequence similarity was detected between the viromes from American and Spanish salterns (18). Some hits were also reported to the described haloviruses, although not on a large scale (18, 91, 92). Because only a fraction of microorganisms are cultivable by current methods, on the level of microbial communities, most of the viruses and potential host organisms remain unknown. However, halophilic archaeal strains belonging to the dominant genera (i.e. *Haloquadratum*, *Halorubrum*) observed in different hypersaline

environments are cultivable (96), but while several haloviruses are known for different strains of *Halorubrum*, none have been isolated for strains belonging to *Haloquadratum* (Tables 1-4). A few years ago, an environmental virus, called EHP-1 was reported in a metagenomic data set from a Spanish saltern (97). This virus candidate, which to date has not been isolated, was suggested to infect *Haloquadratum walsbyi*. The genome sequence of EHP-1 was reported to be 35 kb (97).

7. Conclusions

Haloarchaeal viruses represent the most numerous group of isolated viruses infecting archaea known to date, but are morphologically less diverse than the described crenarchaeal viruses (44). The number of different halovirus architectures is limited to four morphotypes: tailed icosahedral, icosahedral internal membrane containing, pleomorphic, and spindle-shaped viruses (Figs. 1-2). The majority of these virus isolates are tailed icosahedral viruses with contractile tails, reflecting the universality of this morphotype among prokaryotic viruses (Fig. 1b; Fig. 2a-b, and e). It is considered that ~96 % of bacteriophages (from isolates and environmental diversity) represent the tailed icosahedral morphotype of *Caudovirales* viral order while archaeal viruses are morphologically more diverse, characterized by the abundance of different lemon-shaped viruses. Based on TEM studies of hypersaline environments, tailed icosahedral viruses are rare in highly saline waters and their dominance in halovirus isolations is possibly due to biased culturing methods.

Broad host ranges are characteristic to haloarchaeal myoviruses (as for myoviruses of bacteria) of which many are known to infect archaea from various genera (21). In addition to myoviruses, several siphoviruses and one podovirus, HSTV-1, have been described. Recently, HSTV-1 was shown to have the same HK97 MCP fold than tailed icosahedral

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4 bacteriophages and eukaryotic herpes viruses indicating their common ancestry (42).
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6 Haloarchaeal icosahedral internal membrane-containing viruses, on the other hand, are
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8 suggested to belong to the lineage of PRD1-like viruses which also comprises viruses
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10 infecting hosts from all three domains of life. Although no high resolution MCP structures are
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12 yet known for the haloarchaeal icosahedral viruses, cryo-EM studies (Fig. 3) together with
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14 sequence information support their relatedness (33, 40, 71).
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18 To date, only one spindle-shaped virus, His1, has been described for haloarchaea and more
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20 halophilic isolates are desperately needed to better understand this virion architecture that is
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22 considered unique and specific for archaeal viruses. It has been suggested that the short-
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24 tailed spindle-shaped viruses infecting crenarchaea and euryarchaea are related and belong
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26 to the same structure-based lineage.
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30 Haloarchaeal pleomorphic viruses are the first family of related viruses with different genome
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32 types and consequently have different replication strategies. Thus, their discovery has
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34 opened up the world of virus characterization to a more structural perspective instead of
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36 solely relying on replication and host based taxonomy. The close resemblance of these
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38 viruses to membrane vesicles produced by archaea and other microorganisms suggests that
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40 these viruses might have an evolutionary connection with proviruses, plasmids, and vesicles,
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42 as well as with the bacterial vesicular mycoplasmaviruses (88, 90).
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46 Metagenomics and other culture-independent molecular methods are developing fast and
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48 able to explore whole microbial communities, their dynamics, as well as interactions between
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50 virus-like elements and potential host organisms. It remains to be seen whether all the
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52 unusual particle morphologies observed by TEM in hypersaline environments belong to the
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54 virosphere and whether viruses infecting euryarchaea and crenarchaea have more similar
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56 virus morphotypes that shall be discovered in the future.
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4 Although especially the isolations of crenarchaeal viruses have led into the discovery of
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6 several novel virion morphotypes, the overall number of different virus architectures among
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8 the known viruses is still somewhere below 50. Since the isolation of the first haloarchaeal
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10 virus in 1974, several halovirus screenings have been made including two large
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12 environmental studies that have raised the number of known haloarchaeal viruses from less
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14 than 20 to 90 (Fig. 1). Regardless of these efforts, only one novel morphotype, the
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16 pleomorphic viruses, were found. This supports the previous hypothesis of the limited number
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18 of virus morphologies among the $\sim 10^{31}$ viruses (68) on our planet (37). Novel virus
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20 morphotypes will certainly be discovered in the future, but the overall number of structure-
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22 based viral lineages probably remains fairly low.
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31 **Contributions**

32
33 N.S. Atanasova, D.H. Bamford, and H.M. Oksanen planned and wrote the manuscript.
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39
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54 **Conflict of Interest**

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57 None declared.
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Tables

Table 1. Viruses with contractile tails (myoviruses).

No.	Virus	Host	Genome	Reference
1	Hs1	<i>Halobacterium salinarum</i> strain 1	ND	(23, 51)
2	ΦH	<i>Halobacterium salinarum</i> ATCC 29341	59,000 bp	(25, 53, 54)
3	S5100	<i>Halobacterium salinarum</i> NRC 34001	ND	(52)
4	HF1	<i>Haloferrax lucentense</i>	75,898 bp	(59)
5	HF2	<i>Halorubrum coriense</i>	79,700 bp	(59, 98)
6	ΦCh1	<i>Natrialba magadii</i>	58,500 bp	(55, 56, 99)
7	HRTV-1	<i>Halorubrum</i> sp. s1-1	ND	(100)
8	HRTV-2	<i>Halorubrum</i> sp. s1-2	ND	(9)
9	HRTV-3	<i>Halorubrum</i> sp. s5a-2	ND	(9)
10	HRTV-5	<i>Halorubrum</i> sp. S5a-3	76,134 bp	(9, 50)
11	HRTV-6	<i>Halorubrum</i> sp. S5a-4	ND	(9)
12	HRTV-7	<i>Halorubrum</i> sp. B2-2	69,048 bp	(9, 50)
13	HJTV-1	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(9)
14	HJTV-2	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(9)
15	HRTV-8	<i>Halorubrum</i> sp. B2-2	74,519 bp	(9, 50)
16	HGTV-1	<i>Halogramma</i> sp. SS5-1	143,855 bp	(9, 50)
17	HATV-1	<i>Haloarcula</i> sp. SS5-2	ND	(9)
18	HSTV-2	<i>Halorubrum sodomense</i> DSM 33755	68,187 bp	(9, 46)
19	HSTV-3	<i>Halorubrum sodomense</i> DSM 33755	ND	(9)
20	HRTV-9	<i>Halorubrum</i> sp. B2-2	ND	(9)
21	HRTV-10	<i>Halorubrum</i> sp. B2-2	ND	(9)
22	HATV-2	<i>Haloarcula</i> sp. E301-5	ND	(9)
23	HTV-1	Halophilic isolate E200-7	ND	(9)
24	HRTV-11	<i>Halorubrum</i> sp. SL5	ND	(9)
25	HRTV-12	<i>Halorubrum</i> sp. B2-2	ND	(9)
26	HRTV-13	<i>Halorubrum</i> sp. SS8-2	ND	(21)
27	HRTV-14	<i>Halorubrum</i> sp. SS6-2	ND	(21)
28	HRTV-15	<i>Halorubrum</i> sp. SS6-2	ND	(21)
29	HRTV-16	<i>Halorubrum</i> sp. SS6-2	ND	(21)
30	HRTV-17	<i>Halorubrum</i> sp. SS9-12	ND	(21)
31	HRTV-18	<i>Halorubrum</i> sp. SS10-3	ND	(21)
32	HRTV-19	<i>Halorubrum</i> sp. SS10-3	ND	(21)
33	HRTV-20	<i>Halorubrum</i> sp. SS10-9	ND	(21)
34	HRTV-21	<i>Halorubrum</i> sp. SS10-9	ND	(21)
35	HRTV-22	<i>Halorubrum</i> sp. SS10-9	ND	(21)
36	HRTV-23	<i>Halorubrum</i> sp. SS10-9	ND	(21)
37	HRTV-24	<i>Halorubrum</i> sp. SS10-9	ND	(21)
38	HRTV-25	<i>Halorubrum</i> sp. SS13-12	ND	(21)
39	HRTV-26	<i>Halorubrum</i> sp. SS13-13	ND	(21)
40	HRTV-27	<i>Halorubrum</i> sp. SS13-13	ND	(21)
41	HSTV-4	<i>Halorubrum sodomense</i> DSM 33755	ND	(21)
42	HCTV-6	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
43	HCTV-7	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
44	HCTV-8	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
45	HCTV-9	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
46	HCTV-10	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
47	HCTV-11	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
48	HCTV-12	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
49	HCTV-13	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
50	HCTV-14	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
51	HCTV-15	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
52	HJTV-3	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(21)

Table 2. Viruses with non-contractile tails

No.	Virus	Host	Morphology	Genome	Reference
Siphoviruses					
53	Hh1	<i>Halobacterium salinarum</i> ATCC 29341	<i>Siphoviridae</i>	37,200 bp	(25, 62)
54	Hh3	<i>Halobacterium salinarum</i> ATCC 29341	<i>Siphoviridae</i>	29,400 bp	(25, 62)
55	Ja1	<i>Halobacterium salinarum</i> NRC 34001	<i>Siphoviridae</i>	ND	(25, 64)
56	ΦN	<i>Halobacterium salinarum</i> NRL/JW	<i>Siphoviridae</i>	56,000 bp	(25, 61)
57	S45	<i>Halobacterium salinarum</i> NRC 34001	<i>Siphoviridae</i>	ND	(63)
58	B10	<i>Halobacterium</i> sp. B10	<i>Siphoviridae</i>	ND	(101, 102)
59	BJ1	<i>Halorubrum</i> sp. strain BJ1 B11	<i>Siphoviridae</i>	42,271 bp	(65)
60	HRTV-4	<i>Halorubrum</i> sp. SSa-3	<i>Siphoviridae</i>	35,722 bp	(9, 50)
61	HHTV-1	<i>Haloarcula hispanica</i> ATCC 33960	<i>Siphoviridae</i>	49,107 bp	(9, 50, 100)
62	HCTV-1	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	103,257 bp	(9, 50, 100)
63	HVTV-1	<i>Haloarcula vallismortis</i> ATCC 29715	<i>Siphoviridae</i>	101,734 bp	(9, 46)
64	HCTV-2	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	54,291 bp	(9, 50)
65	HCTV-5	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	102,105 bp	(9, 50)
66	HVTV-2	<i>Haloarcula vallismortis</i> ATCC 29715	<i>Siphoviridae</i>	ND	(9)
67	HHTV-2	<i>Haloarcula hispanica</i> ATCC 33960	<i>Siphoviridae</i>	52,643 bp	(9, 50)
68	HRTV-28	<i>Halorubrum</i> sp. SS8-7	<i>Siphoviridae</i>	ND	(21)
69	HRTV-29	<i>Halorubrum</i> sp. SS7-4	<i>Siphoviridae</i>	ND	(21)
70	HATV-3	<i>Haloarcula</i> sp. SS8-5	<i>Siphoviridae</i>	ND	(21)
71	HCTV-16	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	ND	(21)
72	HCTV-17	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	ND	(21)
Podoviruses					
73	HSTV-1	" <i>Haloarcula sinaiensis</i> " ATCC 33800	<i>Podoviridae</i>	32,189 bp	(9, 42)

Table 3. Icosahedral internal membrane-containing viruses

No.	Virus	Host	Genome	Genome type	Reference
74	SH1	<i>Haloarcula hispanica</i> ATCC 33960	30,898 bp	linear dsDNA	(30)
75	HHIV-2	<i>Haloarcula hispanica</i> ATCC 33960	30,578 bp	linear dsDNA	(40)
76	SNJ1	<i>Natrinema</i> sp. J7-2	16,341 bp	circular dsDNA	(33)
77	PH1	<i>Haloarcula hispanica</i> ATCC 33960	28,064 bp	linear dsDNA	(32)
78	HCIV-1	" <i>Haloarcula californiae</i> " ATCC 33799	ND	ND	(21)

Table 4. Pleomorphic and spindle-shaped viruses

No.	Virus	Host	Genome	Genome type	Reference
Pleomorphic viruses					
79	HRPV-1	<i>Halorubrum</i> sp. PV6	7048 nt	circular ssDNA	(31, 86)
80	HHPV-1	<i>Haloarcula hispanica</i> ATCC 33960	8082 bp	circular dsDNA	(87)
81	HRPV-2	<i>Halorubrum</i> sp. SS5-4	10 656 nt	circular ssDNA	(9, 29, 84)
82	HRPV-3	<i>Halorubrum</i> sp. SP3-3	8770 bp	circular dsDNA	(9, 29, 84)
83	HRPV-6	<i>Halorubrum</i> sp. SS7-4	8549 nt	circular ssDNA	(29, 84)
84	HGPV-1	<i>Halogeometricum</i> sp. CG-9	9694 bp	circular dsDNA	(29, 84)
85	His2	<i>Haloarcula hispanica</i> ATCC 33960	16,067 bp	linear dsDNA	(28, 29)
86	HRPV-7	<i>Halorubrum</i> sp. SS5-4	ND	ND	(21)
87	HRPV-8	<i>Halorubrum</i> sp. SP3-3	ND	ND	(21)
88	HAPV-1	<i>Haloarcula</i> sp. SS13-14	ND	ND	(21)
89	HHPV-2	<i>Haloarcula hispanica</i> ATCC 33960	8176 nt	circular ssDNA	(85)
Spindle-shaped viruses					
90	His1	<i>Haloarcula hispanica</i> ATCC 33960	14,464 bp	Linear dsDNA	(28, 80, 81)

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Figure Legends

Figure 1. Distribution of haloarchaeal virus morphotypes from their discovery to current times.

a. Morphotypes of the known haloarchaeal viruses indicated by specific colors (for a., b., and c.): tailed icosahedral viruses with contractile tail (light green, myoviruses), tailed icosahedral viruses with non-contractile tail (light-blue, siphoviruses; blue podoviruses), icosahedral internal membrane-containing viruses (purple), spindle-shaped viruses (pink) and pleomorphic viruses (light-pink). b. Numeric distribution of the known morphotypes. c. Numbers of the isolated haloarchaeal viruses over time.

Figure 2. Transmission electron micrographs of the negatively stained haloarchaeal viruses representing all the known morphotypes. a. Tailed icosahedral virus with a contractile tail (myovirus HRTV-16). b. Tailed icosahedral virus with a non-contractile tail (siphovirus HVTV-1). c. Spindle-shaped virus (His1). d. Icosahedral internal membrane-containing virus (HCIV-1). e. Tailed icosahedral virus with a non-contractile tail (podovirus HSTV-1). f. Pleomorphic virus (HRPV-6). Bar is 100 nm. Viruses were negatively stained with uranyl acetate (a., c., d.), potassium phosphotungstate (b., e.), or ammonium molybdate (f.).

Figure 3. Structures of the haloarchaeal podovirus HSTV-1 and the icosahedral internal membrane-containing virus SH1 based on cryo-EM and 3D reconstruction. a. Central section of the icosahedral reconstruction of HSTV-1 is shown viewed along an icosahedral twofold symmetry axis. Twofold (ellipse), threefold (triangle), and fivefold (pentagon) symmetry axes are indicated (bar is 10 nm). b. 3D isosurface representation of HSTV-1. c. HK97 MCP fold (PDB code 1OHG). d. Averaged density showing the MCP fold of HSTV-1 calculated from the six segmented subunits from one HSTV-1 hexamer. Red ribbon indicates the HK97 chain and

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4 blue ribbon the HSTV-1 MCP, gp14, fitted into the EM density. e. Central section of the
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6 icosahedral reconstruction of SH1 viewed along an icosahedral twofold symmetry axis.
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8 Twofold (ellipse), threefold (triangle), and fivefold (pentagon) symmetry axes are indicated
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10 (bar is 20 nm). Black arrow points at the internal membrane. f. 3D isosurface representation
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12 of SH1. Virions are in scale. a-b and d-f are adapted from (42) and (71). (Copyright© National
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14 Academy of Sciences (USA) [PNAS, doi: 10.1073/pnas.1303047110 (for HSTV-1) and doi:
15
16 10.1073/pnas.0801758105 (for SH1). HK97 MCP fold was created using UCSF Chimera
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Table 1

Table 1. Viruses with contractile tails (myoviruses).

No.	Virus	Host	Genome	Reference
1	Hs1	<i>Halobacterium salinarum</i> strain 1	ND	(23, 51)
2	ΦH	<i>Halobacterium salinarum</i> ATCC 29341	59,000 bp	(25, 53, 54)
3	S5100	<i>Halobacterium salinarum</i> NRC 34001	ND	(52)
4	HF1	<i>Haloferrax lucentense</i>	75,898 bp	(59)
5	HF2	<i>Halorubrum coriense</i>	79,700 bp	(59, 98)
6	ΦCh1	<i>Natrialba magadii</i>	58,500 bp	(55, 56, 99)
7	HRTV-1	<i>Halorubrum</i> sp. s1-1	ND	(100)
8	HRTV-2	<i>Halorubrum</i> sp. s1-2	ND	(9)
9	HRTV-3	<i>Halorubrum</i> sp. s5a-2	ND	(9)
10	HRTV-5	<i>Halorubrum</i> sp. S5a-3	76,134 bp	(9, 50)
11	HRTV-6	<i>Halorubrum</i> sp. S5a-4	ND	(9)
12	HRTV-7	<i>Halorubrum</i> sp. B2-2	69,048 bp	(9, 50)
13	HJTV-1	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(9)
14	HJTV-2	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(9)
15	HRTV-8	<i>Halorubrum</i> sp. B2-2	74,519 bp	(9, 50)
16	HGTV-1	<i>Halogramma</i> sp. SS5-1	143,855 bp	(9, 50)
17	HATV-1	<i>Haloarcula</i> sp. SS5-2	ND	(9)
18	HSTV-2	<i>Halorubrum sodomense</i> DSM 33755	68,187 bp	(9, 46)
19	HSTV-3	<i>Halorubrum sodomense</i> DSM 33755	ND	(9)
20	HRTV-9	<i>Halorubrum</i> sp. B2-2	ND	(9)
21	HRTV-10	<i>Halorubrum</i> sp. B2-2	ND	(9)
22	HATV-2	<i>Haloarcula</i> sp. E301-5	ND	(9)
23	HTV-1	Halophilic isolate E200-7	ND	(9)
24	HRTV-11	<i>Halorubrum</i> sp. SL5	ND	(9)
25	HRTV-12	<i>Halorubrum</i> sp. B2-2	ND	(9)
26	HRTV-13	<i>Halorubrum</i> sp. SS8-2	ND	(21)
27	HRTV-14	<i>Halorubrum</i> sp. SS6-2	ND	(21)
28	HRTV-15	<i>Halorubrum</i> sp. SS6-2	ND	(21)
29	HRTV-16	<i>Halorubrum</i> sp. SS6-2	ND	(21)
30	HRTV-17	<i>Halorubrum</i> sp. SS9-12	ND	(21)
31	HRTV-18	<i>Halorubrum</i> sp. SS10-3	ND	(21)
32	HRTV-19	<i>Halorubrum</i> sp. SS10-3	ND	(21)
33	HRTV-20	<i>Halorubrum</i> sp. SS10-9	ND	(21)
34	HRTV-21	<i>Halorubrum</i> sp. SS10-9	ND	(21)
35	HRTV-22	<i>Halorubrum</i> sp. SS10-9	ND	(21)
36	HRTV-23	<i>Halorubrum</i> sp. SS10-9	ND	(21)
37	HRTV-24	<i>Halorubrum</i> sp. SS10-9	ND	(21)
38	HRTV-25	<i>Halorubrum</i> sp. SS13-12	ND	(21)
39	HRTV-26	<i>Halorubrum</i> sp. SS13-13	ND	(21)
40	HRTV-27	<i>Halorubrum</i> sp. SS13-13	ND	(21)
41	HSTV-4	<i>Halorubrum sodomense</i> DSM 33755	ND	(21)
42	HCTV-6	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
43	HCTV-7	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
44	HCTV-8	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
45	HCTV-9	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
46	HCTV-10	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
47	HCTV-11	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
48	HCTV-12	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
49	HCTV-13	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
50	HCTV-14	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
51	HCTV-15	" <i>Haloarcula californiae</i> " ATCC 33799	ND	(21)
52	HJTV-3	<i>Haloarcula japonica</i> TR1 ATCC 49778	ND	(21)

Table 2

Table 2. Viruses with non-contractile tails

No.	Virus	Host	Morphology	Genome	Reference
Siphoviruses					
53	Hh1	<i>Halobacterium salinarum</i> ATCC 29341	<i>Siphoviridae</i>	37,200 bp	(25, 62)
54	Hh3	<i>Halobacterium salinarum</i> ATCC 29341	<i>Siphoviridae</i>	29,400 bp	(25, 62)
55	Ja1	<i>Halobacterium salinarum</i> NRC 34001	<i>Siphoviridae</i>	ND	(25, 64)
56	ΦN	<i>Halobacterium salinarum</i> NRL/JW	<i>Siphoviridae</i>	56,000 bp	(25, 61)
57	S45	<i>Halobacterium salinarum</i> NRC 34001	<i>Siphoviridae</i>	ND	(63)
58	B10	<i>Halobacterium</i> sp. B10	<i>Siphoviridae</i>	ND	(101, 102)
59	BJ1	<i>Halorubrum</i> sp. strain BJ1 B11	<i>Siphoviridae</i>	42,271 bp	(65)
60	HRTV-4	<i>Halorubrum</i> sp. SSa-3	<i>Siphoviridae</i>	35,722 bp	(9, 50)
61	HHTV-1	<i>Haloarcula hispanica</i> ATCC 33960	<i>Siphoviridae</i>	49,107 bp	(9, 50, 100)
62	HCTV-1	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	103,257 bp	(9, 50, 100)
63	HVTV-1	<i>Haloarcula vallismortis</i> ATCC 29715	<i>Siphoviridae</i>	101,734 bp	(9, 46)
64	HCTV-2	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	54,291 bp	(9, 50)
65	HCTV-5	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	102,105 bp	(9, 50)
66	HVTV-2	<i>Haloarcula vallismortis</i> ATCC 29715	<i>Siphoviridae</i>	ND	(9)
67	HHTV-2	<i>Haloarcula hispanica</i> ATCC 33960	<i>Siphoviridae</i>	52,643 bp	(9, 50)
68	HRTV-28	<i>Halorubrum</i> sp. SS8-7	<i>Siphoviridae</i>	ND	(21)
69	HRTV-29	<i>Halorubrum</i> sp. SS7-4	<i>Siphoviridae</i>	ND	(21)
70	HATV-3	<i>Haloarcula</i> sp. SS8-5	<i>Siphoviridae</i>	ND	(21)
71	HCTV-16	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	ND	(21)
72	HCTV-17	" <i>Haloarcula californiae</i> " ATCC 33799	<i>Siphoviridae</i>	ND	(21)
Podoviruses					
73	HSTV-1	" <i>Haloarcula sinaiensis</i> " ATCC 33800	<i>Podoviridae</i>	32,189 bp	(9, 42)

Table 3

Table 3. Icosahedral internal membrane-containing viruses

No.	Virus	Host	Genome	Genome type	Reference
74	SH1	<i>Haloarcula hispanica</i> ATCC 33960	30,898 bp	linear dsDNA	(30)
75	HHIV-2	<i>Haloarcula hispanica</i> ATCC 33960	30,578 bp	linear dsDNA	(40)
76	SNJ1	<i>Natrinema</i> sp. J7-2	16,341 bp	circular dsDNA	(33)
77	PH1	<i>Haloarcula hispanica</i> ATCC 33960	28,064 bp	linear dsDNA	(32)
78	HCIV-1	" <i>Haloarcula californiae</i> " ATCC 33799	ND	ND	(21)

Table 4

Table 4. Pleomorphic and spindle-shaped viruses

No.	Virus	Host	Genome	Genome type	Reference
Pleomorphic viruses					
79	HRPV-1	<i>Halorubrum</i> sp. PV6	7048 nt	circular ssDNA	(31, 86)
80	HHPV-1	<i>Haloarcula hispanica</i> ATCC 33960	8082 bp	circular dsDNA	(87)
81	HRPV-2	<i>Halorubrum</i> sp. SS5-4	10 656 nt	circular ssDNA	(9, 29, 84)
82	HRPV-3	<i>Halorubrum</i> sp. SP3-3	8770 bp	circular dsDNA	(9, 29, 84)
83	HRPV-6	<i>Halorubrum</i> sp. SS7-4	8549 nt	circular ssDNA	(29, 84)
84	HGPV-1	<i>Halogeometricum</i> sp. CG-9	9694 bp	circular dsDNA	(29, 84)
85	His2	<i>Haloarcula hispanica</i> ATCC 33960	16,067 bp	linear dsDNA	(28, 29)
86	HRPV-7	<i>Halorubrum</i> sp. SS5-4	ND	ND	(21)
87	HRPV-8	<i>Halorubrum</i> sp. SP3-3	ND	ND	(21)
88	HAPV-1	<i>Haloarcula</i> sp. SS13-14	ND	ND	(21)
89	HHPV-2	<i>Haloarcula hispanica</i> ATCC 33960	8176 nt	circular ssDNA	(85)
Spindle-shaped viruses					
90	His1	<i>Haloarcula hispanica</i> ATCC 33960	14,464 bp	Linear dsDNA	(28, 80, 81)

Figure 1
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Figure 2
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Figure 3
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