Mini-review

Viruses of hyperthermophilic Archaea

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Abstract

The viruses of Archaea are likely to be useful tools for studying host evolution, host biochemical pathways, and as tools for the biotechnology industry. Many of the viruses isolated from Archaea show distinct morphologies and genes. The euryarchaeal viruses show morphologies similar to the head-and-tail phage isolated from Bacteria; however, sequence analysis of viral genomes from Crenarchaea shows little or no similarity to previously isolated viruses. Because viruses adapt to host organism characteristics, viruses may lead to important discoveries in archaeal biochemistry, genetics, and evolution.
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1. Introduction

In general, our understanding of the Archaea lags far behind our knowledge of the domains Bacteria and Eukarya. Viruses of Archaea may prove useful for unraveling archaeal biochemistry. Many, but not all, Archaea are well represented in extreme environments, where the extreme condition can be temperature, pH, salinity, or a combination of these environments. The domain Archaea is divided into two major kingdoms, the Euryarchaeota and the Crenarchaeota, and two additional kingdoms, the Korarchaeota and the Nanoarchaeota are under consideration. These groups are defined principally based on 16S rDNA sequences [15,47]. The Euryarchaeota comprise many phylogenetically different groups, but mainly consist of the methanogens, methane-producing organisms, and the extreme halophiles, organisms that inhabit high salt environments. Most Euryarchaeota do not live at high temperatures; however, there are some hyperthermophiles (the orders *Thermococcales* and *Archaeoglobales,* and the organisms *Methanopyrus* and *Methanothermus*). The Korarchaeota branch near the root of the archaeal tree; therefore, they may display novel biological properties

relevant to our understanding of ancient organisms [4]. The Nanoarchaeota are suspected to be small symbiotic organisms that are larger than a virus but smaller than any other described organism. The organisms are only found in combination with other species [17]. The Crenarchaeota are distinctly different from the Euryarchaeota and contain organisms that live at both ends of the temperature spectrum [4]. Most cultured crenarchaeotes are hyperthermophiles that grow optimally above 80 °C. Hyperthermophilic crenarchaeotes have been isolated from both terrestrial and marine environments. The three most studied orders are the anaerobic, sulfur-reducers *Thermoproteales*, the aerobic, sulfur-oxidizers *Sulfolobales*, and the *Desulfurococcales*. The recent completion of several archaeal genomes is raising many questions about archaeal gene structure, expression, and functions [8,11,13, 14,19–22,24,28,37,38,40,42,43]. Clearly, much of archaeal biochemistry remains to be elucidated.

The recent discovery of many novel archaeal viruses, especially among members of the extremely thermophilic Crenarchaeota, is likely to lead to a more complete understanding of not only Archaea, but also the biochemical adaptations required for life in extreme environments, and to new insights into both host and virus evolution. The detailed analysis of viruses often leads to an enhanced understanding of a particular cellular environment because, by definition, viruses are molecular parasites of the cells in which they replicate. Since all viruses are dependent on the host

cell's biochemical machinery for their replication, they are highly adapted to the unique characteristics of their host's cellular environment. The relative ease of both genetic and biochemical analyses of viruses as compared to their more complex hosts makes them very attractive systems for gaining new insights into unique aspects of Archaea.

Archaeal viruses are likely to provide genes and gene products of utility to the biotechnology industry. In recent years, the biotechnology industry has become increasingly interested in viruses that replicate in extreme environments because they are likely sources of commercially useful reagents (e.g., enzymes) that function in unusual chemical and temperature environments as compared to viruses replicating in mesophilic hosts. In addition, archaeal viruses are being developed as vectors for the introduction and expression of homologous and heterologous gene products in Archaea [9,44].

An astonishing feature of many of the newly discovered viruses of the Crenarchaeota is that they are both morphologically and genetically novel. Many of these newly discovered virus particles, such as spindle-shaped and double-tailed morphologies, have not been previously observed in any other virus family. For most of these viruses, analysis of their genomes shows little or no similarity to genes in the public databases. Prior to the discovery of archaeal viruses, 75 families of viruses were recognized, comprising a total of more than 4000 viruses (http://www.ncbi.nlm.nih.gov/ICTV/). As described below, many of the newly discovered archaeal viruses have been recognized as completely new families of viruses and there are certainly many more to be discovered. The field of archaeal virology is quickly proving to be unique and exciting. The objective of this review is to briefly outline our current understanding of archaeal viruses with particular emphasis on viruses from crenarchaeal hosts.

2. Archaeal viruses

When compared to more than 4000 different viruses isolated from Eukarya and Bacteria, relatively few viruses have been isolated from Archaea (Tables 1a and 1b). A total of 36 archaeal viruses or virus-like particles have been identified, but few have been characterized in detail. In addition, further analysis of these 36 archaeal viruses or virus-like particles could reveal that some may be members of the same virus families. The lack of archaeal viruses described to date is most likely due to the limited effort in isolating archaeal viruses and not a reflection of any biological restriction limiting viral diversity and number in the Archaea. All the archaeal viruses isolated to date are DNA viruses (excluding φCh1 isolated from *Natrialba* species which may contain an RNA component [23,46]). Archaeal viruses contain circular or linear double-stranded (ds) genomes that range in size from 12–230 kb (kilobase) pairs. The complete or nearly complete genome sequence has only been determined for

seven of these viruses [3,23,27,29,31,32,45]. Most viruses of the Euryarchaeota show morphologies similar to viruses that infect organisms within the domain Bacteria, suggesting that they may share common ancestors. The head-and-tail viruses isolated from methanogens and halophiles belong to the viral families *Myoviridae* and *Siphoviridae* [1], excluding two viruses, His1, a virus recently found that might belong to the *Fuselloviridae* family [5], and a virus-like particle isolated from *Methanococcus voltae* [48]. The International Committee on Taxonomy of Viruses (ICTV) classifies two halophilic viruses in the *Myoviridae* family, and three methanogenic viruses in the *Siphoviridae* family (http://www.ncbi.nlm.nih.gov/ICTV/). The other Euryarchaeota viruses have not been officially classified. In contrast, the viruses isolated from crenarchaeal hosts are morphologically distinct. Some have completely novel morphologies not seen in other viruses of Archaea, Bacteria or Eukarya.

The isolation of archaeal viruses from extreme environments is often problematic. Unlike the recent detection of abundant viruses from marine environments by direct filtration of environmental samples [10,41], direct isolation of archaeal viruses from extreme environments by filtration has not been successful [36]. The reason for this failure may be because these viruses may have evolved to minimize the time that they exist free of their hosts in hostile chemical environments. Another potential limitation to isolation is that few potential archaeal hosts have been cultivated or those that have been cultured require conditions that are not conducive to the isolation of viruses. For example, true plaque assays for isolation of viruses from high temperature Crenarchaeota have been difficult to develop because of the difficulty of growing host lawns on solid media at temperatures >75 °C, and the fact that the majority of crenarchaeal viruses described are not lytic. However, successful plaque-like growth-inhibition assays for the detection of *Sulfolobus* viruses on lawns of *Sulfolobus* hosts grown on Gelrite^TM plates have been developed [51]. However, not all growth inhibition zones are the result of virus replication. For example, anti-archaeal compounds (termed sulfolobicins) produced by some *Sulfolobus* strains limit the growth of related *Sulfolobus* strains [33]. This inhibition of growth sometimes appears indistinguishable from virus-induced growth inhibition zones. In addition, the fact that many of the archaeal viruses (especially of the Crenarchaeota) are unrelated, with respect to morphology, to previously described viruses makes it more difficult to depend on previously established purification protocols for viruses and phage from Eukarya and Bacteria. Similarly, unlike ribosomal RNA gene sequences, there are no known universal nucleic acid signatures for viruses, making identification based on nucleic acid hybridization impossible. Given these limitations it is no surprise that most archaeal viruses have been discovered to date by large scale random screening [51], screening potential hosts that can be cultured for extra chromosomal ele-

Table 1a
Characteristics of viruses from Euryarchaeota[a]

Virus name	Genome	Morphology	Host
			Extreme halophiles[b]
φH	Linear 59-kb DNA	Head (64 nm) and tail (170 nm)	*H. halobium*
φN	Linear 56-kb DNA	Head (55 nm) and tail (80 nm)	*H. halobium*
HF1	Linear 73.5-kb dsDNA	Head (58 nm) and tail (94 nm)	*Haloarcula* spp.
			Haloferax volcanii
			H. salinarium
HF2	Linear 79.7-kb dsDNA	Head (58 nm) and tail (94 nm)	*H. saccharovorum*
S45	Linear dsDNA	Head (40 nm) and tail (70 nm)	*Halobacterium* spp.
His1	Linear 14.9-kb dsDNA	Lemon-shaped (74 × 44 nm)	*Haloarcula hispanica*
			H. cutirubrum
Hs1	Data not known	Head (50 nm) and tail (120 nm)	*H. salinarium*
Ja1	Linear 230-kb dsDNA	Head (90 nm) and tail (150 nm)	*H. cutirubrum*
			H. salinarum
Hh-1	37.2-kb dsDNA	Head (60 nm) and tail (100 nm)	*H. halobium*
Hh-3	29.4-kb dsDNA	Head (75 nm) and tail (50 nm)	*H. halobium*
			H. cutirubrum
φCh1	Linear 55-kb dsDNA	Head (70 nm) and tail (130 nm)	*Natrialba magadii*
	Small RNAs		
			Methanogens[c]
ψM1	Linear 30-kb dsDNA	Head (55 nm) and tail (210 nm)	*M. thermoautotrophicum*
			strain Marburg
ψM2	Linear 26-kb dsDNA	Head (55 nm) and tail (210 nm)	*M. thermoautotrophicum*
			strain Marburg
φF1	Linear 85-kb dsDNA	Head (70 nm) and tail (160 nm)	*M. thermoformicium*
			M. thermoautotrophicum
φF3	Circular 36-kb dsDNA	Head (55 nm) and flexible tail (230 nm)	*M. thermoautotrophicum*
PG	50-kb dsDNA	Head and tail	*Methanobrevibacter smithii* strain G
PSM1	35-kb dsDNA	Head and tail	*Methanobrevibacter smithii*
VTA	Data not known	Head (40 nm) and tail (61 nm)	*Methanococcus voltae*

[a] Modified from Arnold et. al. 1999;   [b] *H.* = *Halobacterium*;   [c] *M.* = *Methanobacterium*.

Table 1b
Characteristics of hyperthermophilic viruses and virus-like particles from Crenarchaeota[a]

Virus name	Genome	Morphology	Host
			Thermoproteus
TTV1	Linear 15.9-kb dsDNA	Flexible rod (40 × 400 nm)	*T. tenax*
TTV2	Linear 16-kb dsDNA	Filamentous (20 × 1250 nm)	*T. tenax*
TTV3	Linear 27-kb dsDNA	Filamentous (30 × 2500 nm)	*T. tenax*
TTV4	Linear 17-kb dsDNA	Stiff rod (30 × 500 nm)	*T. tenax*
			Sulfolobus
SSV1	ccc 15.5-kb dsDNA	Lemon-shaped (90 × 60 nm)	*S. shibatae*
			strain B12
SSV2	ccc 14.8-kb dsDNA	Lemon-shaped (80 × 55 nm)	*S. islandicus*
SSV3	ccc 15-kb dsDNA	Lemon-shaped (80 × 55 nm)	*S. islandicus*
SSV-RH[b]	ccc ~16-kb dsDNA	Lemon-shaped	*S. solfataricus*
SSV-GV[b]	ccc ~17-kb dsDNA	Lemon-shaped	*S. solfataricus*
SNDV	ccc 20-kb dsDNA	Bearded droplet (145 × 75 nm)	*Sulfolobus* sp.
SIFV	linear 41-kb dsDNA	Filamentous (1950 × 24 nm)	*S. islandicus*
SIRV1	Linear 32.3-kb dsDNA	Stiff rod (780 × 23 nm)	*S. islandicus*
SIRV2	Linear 35.5-kb dsDNA	Stiff rod (900 × 23 nm)	*S. islandicus*
SIRV-YNP[b]	Linear 33-kb dsDNA	Stiff rod (900 × 25 nm)	*S. solfataricus*
DAFV	Linear 56-kb dsDNA	Filamentous (2000 × 27 nm)	*Desulfurolobus* (*Acidianus*) *ambivalens*
LITV[b]	dsDNA	Icosahedral (72 nm)	*S. solfataricus*
SIFV-YNP[b]	Linear 40-kb dsDNA	Filamentous (900–1500 × 50 nm)	*S. solfataricus*
SITV[b] VLP	12-kb dsDNA	Spherical (32 nm)	*S. acidocaldarius*
Double-tailers[b] VLP	dsDNA	Assymmetrical spindle-shaped (100 × 400 nm)	*S. solfataricus*

[a] Modified from Arnold et al. 1999;   [b] not accepted names of viruses.

ments [51], or by direct visualization of concentrated supernatants of enrichment cultures with an electron microscope [1,36,51].

3. Viruses of the Crenarchaeota

3.1. Sulfolobus viruses

Most viruses isolated to date from crenarchaeal hosts have been from the *Sulfolobales* [50–52]. This is mostly due to the relative ease of isolation and culturing of the aerobic *Sulfolobales* as compared to anaerobic members of the crenarchaeotes. *Sulfolobales* is comprised of acidophilic hyperthermophiles that optimally reproduce in a pH range from 1–5 [15] and a temperature range from 60–90 °C [15]. Thermal environments around the world that contain these habitats are likely to contain *Sulfolobus* species. Viruses are commonly found associated with *Sulfolobus* species that have been isolated from thermal acidic features in the United States [36], Japan [49], Iceland [51], Russia (manuscript in preparation), and New Zealand [2]. For example, 22 of 51 *Sulfolobus* enrichment cultures established from diverse locations within Yellowstone National Park USA (YNP) contained one or more distinct virus morphologies [36].

The *Fuselloviridae* (SSVs) are an expanding group of viruses that have been commonly isolated from *Sulfolobus* enrichment cultures [36,50]. SSV1, originally isolated from Beppu, Japan [25], is the type member of the *Fuselloviridae* family and has been the most extensively studied virus from the Crenarchaeota. SSV1 replicates to high copy number [16] and forms 60×90 nm spindle-shaped particles (see Fig. 1A) that assemble and bud out from the host membrane [25]. Extending from one end of the spindle are short tail fibers that are presumably involved with attachment of the virus particles to the *Sulfolobus* cell membrane or its receptor. The morphology of this virus is similar to the morphology of His1, a virus infecting the extremely halophilic archaeon *Haloarcula hispanica* [5], and a virus-like particle from *Methanococcus voltae* [48]. This is the first example of a common viral morphology between the Euryarchaeota and the Crenarchaeota.

SSV1 particle production in culture can be induced by UV irradiation [25] or treatment with mitomycin C [16,49]. The induction of the virus does not result in host cell lysis [16,49]. It has not yet been established if other SSVs can also be induced by irradiation or chemical treatment.

All SSVs have circular dsDNA genomes ranging in size from 14.8 kb to 17 kb (Table 1b, see Fig. 2) and encode for approximately 35 open reading frames (ORFs). In general, the ORFs are tightly arranged on the genome. There appear to be few non-coding regions. The vast majority of these ORFs show little or no similarity to other genes in the public databases. Even when genomes of four SSVs from very distant locations are compared, there are some ORFs which show only limited similarity between the isolates

(manuscript in preparation). Only four of the SSV1 ORFs have assigned functions (see Fig. 1). SSV1 encodes an integrase for insertion of its genome into a tRNA^{ARG} gene in the host *Sulfolobus* genome [29]. Three other ORFs encode the viral structural proteins (VP1, VP2, and VP3). Only SSV1 contains all three viral proteins. Other SSV isolates from Iceland, Russia, and the USA contain VP1 and VP3, but lack VP2 (manuscript in preparation). No virally encoded polymerase has been assigned.

Three different forms of the SSV genome are thought to co-exist in an infected cell. In addition to the integrated copy, both positively and negatively supercoiled episomal forms of the genome are present [26]. The biological function of each form is not known, but it is tempting to speculate that the integrated form is used as a template for transcription, the positively supercoiled form is used for encapsidation within the virus particle, and the negatively supercoiled form used as a template for genome replication.

We only have limited information on SSV gene expression. Nine major transcripts have been detected and mapped onto the SSV1 genome [35]. The nine major transcripts start from six promoter sites. The major transcripts are T1 and T2, which encode for the virus structural proteins; T3 encodes a291 and is stronger than T4; T4 encodes for the largest ORF, c792; and T5 transcribes the integrase gene. T1 is the major transcript produced during exponential growth, and it is increased two-fold when the cells are approaching stationary phase. T2, T3, T4, T7, and T8 are found in five-fold excess during stationary phase [29]. The elucidation of the details of SSV replication cycle remains to be determined. However, it is likely that such an understanding will provide insight into *Sulfolobus* biochemistry, particularly with regard to biochemical mechanisms and control of DNA replication and macromolecular assembly processes in *Sulfolobus*.

Sulfolobus cells can be successfully transfected with SSV1 DNA by electroporation [39]. *S. solfataricus* strain P1, which is a close relative of *S. shibatae*, was transfected with naked SSV1 DNA with a survival rate of 50% and a transfection frequency of 10^{-4} to 10^{-5}. The transfected cells form growth-inhibition zones on virus-free lawns of host cells [39].

SSVs could prove to be very useful tools in studying the genetics of *Sulfolobus*. The SSV1 virus has been used to create a number of shuttle vectors [44]. By a serial selection technique pBluescript S/K$^+$ (Stratagene, La Jolla, CA) was inserted into a nonessential ORF in the SSV1 genome. This construct will replicate in both *Sulfolobus* and *E. coli* and has been found to accommodate relatively large DNA inserts [44]. The chimeric DNA is packaged within virus particles and readily spreads within a culture leading to nearly all cells expressing the vector. Since the virus can be induced with UV irradiation and can be present at a high copy number, it is very appealing for complementation and expression studies.

The *Sulfolobus islandicus* rod-shaped viruses (SIRV) are the second most common viruses isolated from *Sulfolobus*
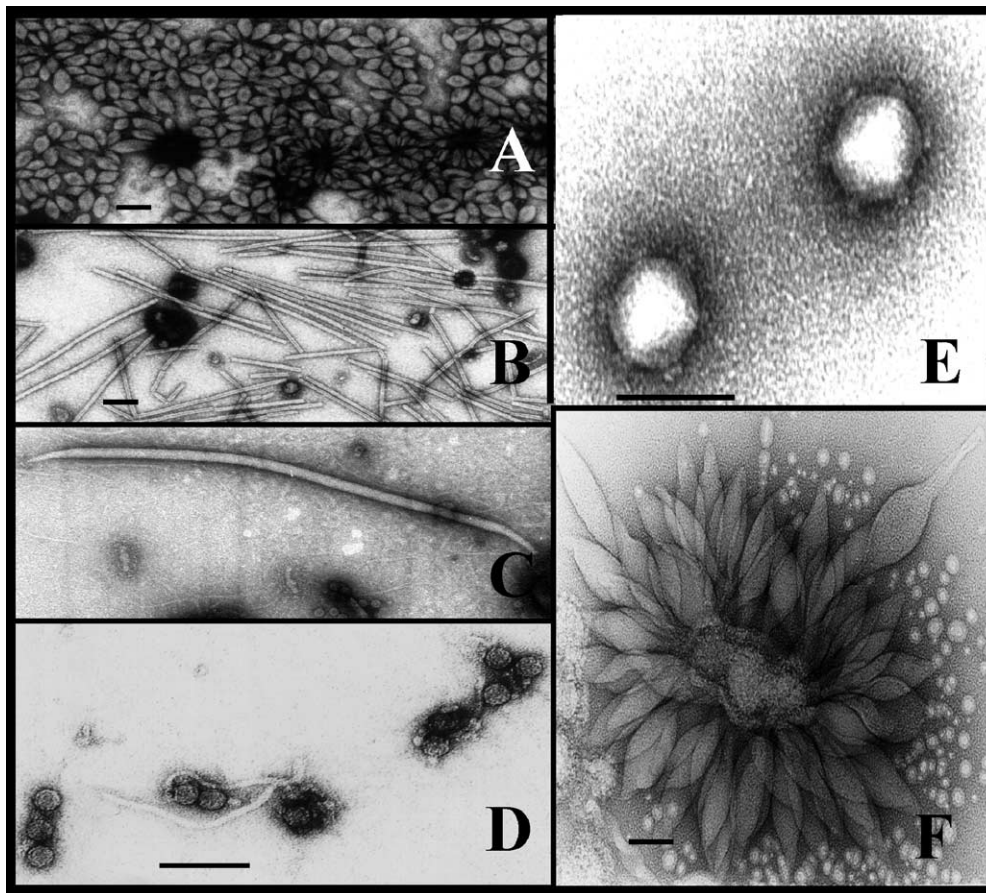
Fig. 1. Examples of viruses isolated from enrichment cultures from Yellowstone National Park. (A) SSV-like virus; (B) SIRV-like virus; (C) SIFV-like virus; (D) 32 nm spherical virus-like particles; (E) 72 nm icosahedral virus; and (F) 100 × 400 nm oblong virus-like particle with tails (modified from [36]).
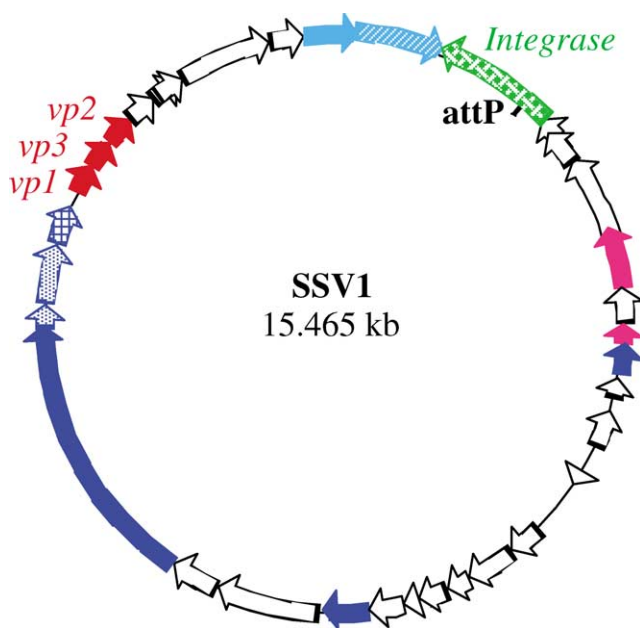

Fig. 2. Genetic map of the SSV1 genome showing 34 open reading frames. attP indicates the site for integration into the *Sulfolobus* genome. VP1, VP3, and VP2 indicate the viral coat proteins. The blue ORFs are essential, and the pink ORFs are not essential (modified from 40).

species. Often the virus is found in enrichment cultures that also contain SSVs [36], potentially due to SIRV induction of SSV [32]. The *Rudiviridae* viral family was created for the SIRV viruses [32]. SIRVs have been isolated from both Iceland and YNP [32,36]. The SIRV viruses are non-enveloped, 23×800–900 nm stiff rods (see Fig. 1B) that contain a circular, single-stranded DNA that is completely self-complementary and functionally can be viewed as double-stranded linear DNA, ends of which are covalently closed. Therefore, the genome is functionally 33–36 kb of dsDNA except for short (4 nucleotide) turns at each end of the dsDNA [7]. The viral DNA genome is coated with a DNA binding protein (which can be considered a viral coat protein) that results in a hollow helical virion with a periodicity of 4.3 nm [32]. Each terminus of the helix is plugged and the virus has tail fibers at both ends [32]. Thus far, there is no indication that the isolated viral genome is infectious (D. Prangishvili, personal communication). UV irradiation or mitomycin C treatment does not induce SIRV production and lysis of the host cells does not occur [32]. Many cultures of *Sulfolobus* infected with a SIRV virus will cure themselves of the virus after numerous transfers [32,36]. The genome of SIRV1 is very unstable.

Upon propagation of this virus in novel host cells, the viral genome of SIRV1 changes dramatically; however, SIRV2 does not appear to have this ability [32]. The terminal regions of SIRV DNA are very similar to DNA structures found in some eukaryal viruses [7], and therefore might have a similar replication strategy. The formation of head-to-head and tail-to-tail linked viral DNA was found to be present in virus-infected cells [30]. It is hypothesized that SIRV DNA replicates through a Holliday junction intermediate. A Holliday junction resolvase that shares homology with other archaeal Holliday junction resolvases has been found in SIRV1 and SIRV2 [6].

The genomes of SIRV1 and SIRV2 from Iceland have been determined [30]. The genomes of the two viruses are very similar. SIRV1 contains 45 ORFs and SIRV2 contains 54 ORFs ranging from 55–1070 amino acids in length. Forty-four ORFs are homologous between the two viruses. Twelve of the shared homologous ORFs and three ORFs from SIRV2 show homology to SIFV ORFs. Twenty-one of the ORFs showed a very limited similarity to eukaryal viral genes. Fourteen were distantly similar to *Poxiviridae* genes and eight were similar to *Amsacta moorei* entomopoxvirus. Finally, there were seven possible homologs to African swine fever virus and *Chorella* viruses [30]. In general, like other Crenarchaeotal viruses, the SIRV genes are unique. However, the distant similarity with eukaryal viral genes suggests a possible common origin for all of these viruses.

A third group of viruses of *Sulfolobus* is poorly understood. The *Lipothrixviridae* family contains the *S. islandicus* filamentous virus (SIFV) and *Desulfurolobus (Acidianus) ambivalens* filamentous virus (DAFV) [3]. SIFV, like SIRV, was originally isolated from Icelandic solfataric fields [3], but since has been discovered in samples obtained from YNP [36]. SIFV is an enveloped, flexible, filamentous virus with mop-like structures at the ends (see Fig. 1C) [3]. SIFV does not seem to cause lysis of the host cells upon infection, and it does not integrate into the host genome. It appears to be maintained in the host cell in an unstable carrier state [3]. The viral genome consists of linear dsDNA of approximately 43 kb. It is possible that the SIFV virus genome termini have similar DNA structure to the SIRV viruses, but that has not been proven as of yet. The average ORF size in SIFV is 500 bp and 90% of the genome is coding sequence [3]. The two major proteins are transcribed in the same direction in a tandem array.

A fourth group of *Sulfolobus* viruses belongs to the proposed *Guttaviridae* family and consists of only one virus, *Sulfolobus neozealandicus* droplet-shaped virus (SNDV), originally isolated from Steaming Hill in New Zealand [2]. The genome of SNDV was found to be approximately 15 kb of double-stranded circular, covalently closed (ccc) DNA [2]. Although the genome size of SNDV is similar to SSV1, there are many distinguishing characteristics. SNDV persists in the host cell in a carrier state and is not integrated, it has a beehive-like ribbed surface, and it has many long tail fibers at its pointed end [2]. SNDV is the only

crenarchaeotal virus that has been shown to have a highly modified genome [2]. Modification of a viral genome has been shown in the euryarchaeotal virus, ϕCh1 [46], but it had not been observed in crenarchaeal viral genomes until the discovery of SNDV.

3.2. Viruses from other Crenarchaeota

Novel viruses have been identified associated with *Thermoproteus* cultures. *Thermoproteus* is a chemolithoautotrophic organism that belongs to the *Thermoproteales* family within the Crenarchaeota [12,53]. *Thermoproteus* thrives in extremely thermophilic environments with temperatures of greater than 88 °C [53]. It is an anaerobic sulfur-reducer living at slightly acidic pH [53].

Thermoproteus tenax was originally isolated from a mud hole in Iceland, and was found to harbor four viruses, TTV1, TTV2, TTV3, and TTV4 (Table 1b) [18]. The four viruses isolated from *T. tenax* each contain linear dsDNA ranging in size from 15.6 kb to 27 kb [18]. DNA sequence homology has not been found between the genomes of the four *Thermoproteus* viruses based on hybridization studies [1]. TTV1, TTV2, and TTV3 appear to belong to the viral family *Lipothrixviridae*, TTV4 has not been assigned to a viral family.

The four *T. tenax* viruses differ in their morphology, protein composition, and lipid composition. The best-characterized *T. tenax* virus is TTV1. The virus is temperate and lysis is induced when sulfur in the culture is consumed [18]. TTV1 is a rod-shaped virus that has rounded protrusions at each end of the virion [18]. TTV1 has a 16-kb linear dsDNA genome that is bound by two highly basic proteins. This central core is surrounded by an inner lipid envelope, which is then covered by a second envelope consisting of host lipids [18]. TTV2 and TTV3 are morphologically similar to each other. Both are flexible (1200–2500 × 2–3 nm) filaments composed of linear DNA surrounded by protein and an outer lipid envelope. *T. tenax* is lysogenic for TTV2 [18]. TTV4 is similar to TTV1 in shape, but TTV4 is highly virulent and causes rapid lysis of the host cells [18]. In general, this group of viruses has been difficult to study mostly because of the difficulty in maintaining the virus in culture.

4. Newly discovered viruses and virus-like particles

Three of the types of viruses isolated from YNP have similar morphologies to viruses previously isolated from Iceland, or Japan [36]. However, several new viruses have been isolated from YNP [36]. At least three new virus morphologies have been observed in *Sulfolobus* enrichment cultures. These include a small 32 nm spherical virus-like particle (see Fig. 1D), a larger 72 nm icosahedral virus with 9 nm projections extending from each of the five-fold vertices of the particle surface (see Fig. 1E), and a 100 ×
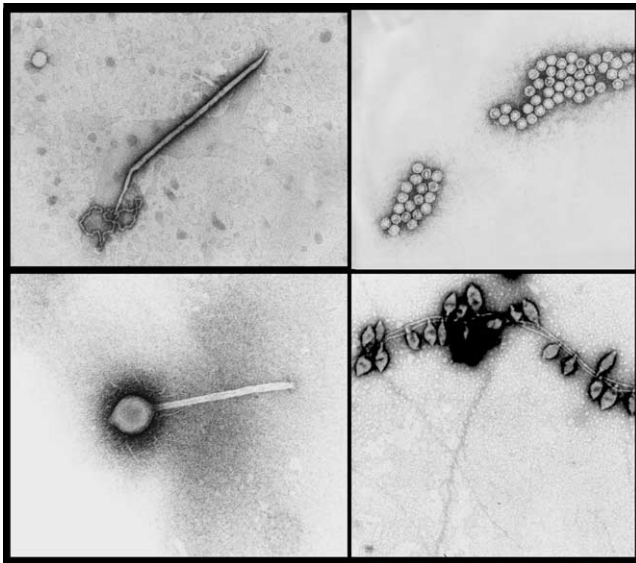
Fig. 3. Examples of virus-like particles visualized in enrichment cultures from Yellowstone National Park.

400 nm oblong virus-like particle with tails (see Fig. 1F) projecting from each end. All of these viruses appear to have DNA genomes and the large icosahedral virus has been shown to infect host cells [36].

New virus-like particles of Crenarchaeota not associated with *Sulfolobus* species have also been isolated (see Fig. 3). Numerous virus morphologies found in *Acidianus* enrichment cultures isolated from YNP have been identified [34]. These include a filamentous virus-like particle morphologically similar to that of SIFV and various head-and-tail morphologies [34].

Novel virus-like particles have also been observed from suspected hyperthermophilic *Thermoproteales* organisms isolated from YNP. The morphologies of the virus-like particles observed in *Thermoproteus* and *Thermophilum* enrichment cultures include SSV-like morphologies (see Fig. 3) that in many cases appear to be budding out from the host cell, and SIRV-like morphologies (W. Zillig, personal communication).

5. Conclusions and future work

Viruses are likely to be a common feature of the archaeal domain. The viruses of Archaea, especially of the crenarchaeal hosts, appear to be quite novel. This suggests that a detailed understanding of these viruses will lead to new insights into archaeal biochemistry, genetics, and evolution. Examination of these viruses is a new field that will likely expand with more discovery and characterization of Archaea.

Some of the major challenges facing archaeal virologists are to unravel the regulation of replication and gene expression of these viruses. At present, we have only an elementary understanding of how these viruses replicate and in-

teract with their host's biochemical machinery. A first step toward this goal will be determining the complete genome sequences as well as determining viral gene functions of these viruses. This task will require multiple approaches using the tools of cell biology, molecular biology and biophysics. A thorough genetic analysis of viral genomes and the effect on host gene expression is required. Research is underway to determine the high-resolution structure of the viral particles as well as the structures of their encoded gene products. This will likely provide new insights into their function. It is likely that such analysis will lead to major discoveries in archaeal biology.

Acknowledgements

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