

# The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life

George Rice<sup>\*†</sup>, Liang Tang<sup>‡</sup>, Kenneth Stedman<sup>§</sup>, Francisco Roberto<sup>¶</sup>, Josh Spuhler<sup>\*†</sup>, Eric Gillitzer<sup>||</sup>, John E. Johnson<sup>‡</sup>, Trevor Douglas<sup>\*†\*\*</sup>, and Mark Young<sup>\*†||††</sup>

\*Thermal Biology Institute and Departments of <sup>†</sup>Microbiology, <sup>\*\*</sup>Chemistry and Biochemistry, and <sup>||</sup>Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717; <sup>‡</sup>Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037; <sup>§</sup>Biology Department, Portland State University, Portland, OR 97207; and <sup>¶</sup>Biotechnology Department, Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID 83415

Communicated by George Bruening, University of California, Davis, CA, March 12, 2004 (received for review December 2, 2003)

Of the three domains of life (Eukarya, Bacteria, and Archaea), the least understood is Archaea and its associated viruses. Many Archaea are extremophiles, with species that are capable of growth at some of the highest temperatures and extremes of pH of all known organisms. Phylogenetic rRNA-encoding DNA analysis places many of the hyperthermophilic Archaea (species with an optimum growth  $\geq 80^\circ\text{C}$ ) at the base of the universal tree of life, suggesting that thermophiles were among the first forms of life on earth. Very few viruses have been identified from Archaea as compared to Bacteria and Eukarya. We report here the structure of a hyperthermophilic virus isolated from an archaeal host found in hot springs in Yellowstone National Park. The sequence of the circular double-stranded DNA viral genome shows that it shares little similarity to other known genes in viruses or other organisms. By comparing the tertiary and quaternary structures of the coat protein of this virus with those of a bacterial and an animal virus, we find conformational relationships among all three, suggesting that some viruses may have a common ancestor that precedes the division into three domains of life  $>3$  billion years ago.

The isolation and characterization of viruses often lead to new insights into virus relationships and to a more detailed understanding of the biochemical environment of their host cells. To date, of the  $\approx 5,100$  known viruses, only 36 have been isolated from Archaea [International Committee on Taxonomy of Viruses, [www.ncbi.nlm.nih.gov/ICTV](http://www.ncbi.nlm.nih.gov/ICTV) (1)]. Viruses and virus-like particles have been isolated from high-temperature ( $\geq 80^\circ\text{C}$ ) acidic pH ( $< 3.0$ ) terrestrial environments throughout the world (2–4). Typically, these environments are found in thermal features such as boiling hot springs, mud pots, fumaroles, and geysers, with a wide range of geochemical compositions (5, 6). Most of the viruses isolated thus far replicate in hyperthermophilic hosts that belong to the Sulfolobales family within the kingdom Crenarchaeota of the domain Archaea (7). To date, these viruses have been unique both in their genome sequence and virion morphology (8). Four previously uncharacterized viral families, the Rudiviridae, Lipothrixviridae, Fuselloviridae, and Guttaviridae, have been created to reflect the unprecedented nature of these viruses. Members of the family Fuselloviridae are characterized by their  $60 \times 90$ -nm spindle-shaped virions that possess an  $\approx 15.5$ -kb circular double-stranded DNA (dsDNA) genome with  $\approx 35$  ORFs (9, 10). Members of the family Rudiviridae have virions that are stiff  $23 \times 800$ - to  $900$ -nm helical rods possessing linear dsDNA genomes (11). *Sulfolobus islandicus* filamentous virus (SIFV) is a member of the family Lipothrixviridae, which is characterized by  $24 \times 1,980$ -nm flexible rods with putative attachment fibers at both ends. The linear dsDNA genome of SIFV is estimated to be 42 kb in length with  $\approx 74$  ORFs (12). A new virus of *Acidianus*, *Acidianus* filamentous virus 1 (AFV1) (13), is an enveloped  $900 \times 24$ -nm filamentous virus with claw-like structures at each end. The 21-kb dsDNA AFV1 genome contains 40 ORFs. Surprisingly, very few ORFs

from these viral genomes have any significant identity to sequences available in the public databases. No high-temperature viruses from Archaea have been analyzed in structural detail.

We report here the structural and genetic characterization of an icosahedral virus from a hyperthermo-acidophilic archaeon isolated from a hot spring in Yellowstone National Park (YNP).

## Materials and Methods

**Virus Isolation.** Enrichment cultures were established from a YNP acidic hot spring (pH 2.9–3.9,  $72$ – $92^\circ\text{C}$ ) in the Rabbit Creek thermal area ( $44^\circ 31.287'\text{N}$ ,  $110^\circ 48.647'\text{W}$ ) within the Midway Geyser Basin, as described (4). A single-colony isolate of *Sulfolobus solfataricus* expressing a spherical virus-like particle was established by previously described methods (4). This isolate was designated YNPRC179.

**Virus Purification.** Isolate YNPRC179 was inoculated into medium 88 ([www.dsmz.de/species/gn301555.htm](http://www.dsmz.de/species/gn301555.htm)), and the culture supernatants were monitored for virus production by direct particle counts with a transmission electron microscope (TEM), as described (4). When virus titers reached maximum levels (typically 3 days), cells were removed by centrifugation ( $6,000 \times g$  for 10 min) and the supernatants filtered through Acrodisc PF 0.8/0.2- $\mu\text{m}$  filters (Pall Gelman Laboratory, Ann Arbor, MI). Filtrate was concentrated by passage through Amicon 100 membranes (Millipore) until the retained volume was  $\approx 8$  ml. The retentate was subsequently loaded onto  $\text{Cs}_2\text{SO}_4$  (38% wt/vol in 25 mM citrate buffer, pH 3.5) and centrifuged at  $247,600 \times g$  for 20 h. Virus bands were removed, placed in 14,000  $M_r$  cutoff dialysis tubing and dialyzed against 25 mM citrate buffer (pH 3.5). Virus was concentrated further with a Microcon 100 (Millipore) when necessary. The resulting virus preparation was characterized by TEM, dynamic light scattering (90 Plus Particle Size Analyzer, Brookhaven Instruments, Holtsville, NY), UV/visible spectroscopy (Agilent 8453, Agilent Technologies, Palo Alto, CA), and SDS/PAGE.

**Cloning and Sequencing of the Viral Genome.** DNA was extracted from purified virus by standard phenol, chloroform, and Proteinase K plus SDS methods (14). Separate restriction endonuclease libraries were constructed by digestion of the extracted DNA with *EcoRI*, *PstI*, and *HindIII*, ligated into P Bluescript II

Abbreviations: dsDNA, double-stranded DNA; YNP, Yellowstone National Park; TEM, transmission electron microscope; MALDI, matrix-assisted laser desorption ionization; STIV, *Sulfolobus* turreted icosahedral virus.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AY569307).

See Commentary on page 7495.

<sup>††</sup>To whom correspondence should be addressed. E-mail: [myoung@montana.edu](mailto:myoung@montana.edu).

© 2004 by The National Academy of Sciences of the USA

plasmid vector (Stratagene), and transformed into ultracompetent XL2-Blue cells (Stratagene) by using the manufacturer's protocols ([www.stratagene.com/lit/manuals.aspx](http://www.stratagene.com/lit/manuals.aspx)). A shotgun library was created by Lucigen (Middleton, WI). Plasmids were purified from the libraries and used as templates for BIG DYE 3.0 chemistry sequencing reactions as described by the manufacturer (Applied Biosystems). DNA sequencing was performed on an ABI 3700 automated DNA sequencer according to manufacturer's protocols (Applied Biosystems). Sequence assembly was performed with SEQUENCHER, Ver. 4.1.2 (Gene Codes, Ann Arbor, MI), and putative ORFs were identified by using NCBI ORF FINDER ([www.ncbi.nlm.nih.gov/gorf/gorf.html](http://www.ncbi.nlm.nih.gov/gorf/gorf.html)). Sequence alignments were performed with BLASTP at the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)), TBLASTN, and BLASTN in BIOLOGY WORKBENCH (<http://workbench.sdsc.edu>). Predicted viral ORFs were compared in the codon usage database ([www.kazusa.or.jp/codon](http://www.kazusa.or.jp/codon)) to those of *Sulfolobus* for codon bias.

**Identification of the Major Virion Protein.** Purified virus was denatured and separated on 12% SDS/PAGE gels, stained with Coomassie brilliant blue R250 (Sigma), and the major protein band excised. The gel slice was cut into smaller pieces, destained by using 50% acetonitrile in 25 mM ammonium bicarbonate, pH 7.9, and desiccated. Ten to fifteen microliters of sequencing grade trypsin (15 ng/ $\mu$ l; Promega) in 25 mM ammonium bicarbonate, pH 7.9, was added to the dehydrated gel slices and allowed to incubate overnight at 37°C. The digestion was stopped by the addition of 20  $\mu$ l of 50% acetonitrile in 25 mM ammonium bicarbonate, pH 7.9, with 1% trifluoroacetic acid (TFA). The matrix for matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) was prepared by mixing twice-recrystallized  $\alpha$ -cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% TFA. A volume of 0.5  $\mu$ l of sample and 0.5  $\mu$ l of matrix was spotted onto a MALDI-TOF plate and allowed to dry. Spectra were collected by using a Bruker Biflex III MALDI-TOF mass spectrophotometer (Bruker Daltonics, Billerica, MA) and calibrated by using tryptic digest fragments. Results were compared with theoretical trypsin digests of viral ORFs by using PEPTIDE MASS at ExPASy (<http://us.expasy.org/tools/peptide-mass.html>).

**Infectivity Assays.** Infectivity assays were carried out by inoculating early log cultures of *S. solfataricus* strains P1 (15) and P2 (16) with 20  $\mu$ l of purified virus. Purified virus was treated separately with either 10% Proteinase K (Ambion, Austin, TX) heated to 55°C for 30 min, 25% chloroform at 22°C for 5 min, 0.3% Triton X-100 at 22°C for 3 min, or 0.1% SDS at 22°C for 3 min. Control cultures were inoculated with untreated purified virus. All infection assays were monitored every 24 h for virus accumulation by TEM. Virion stability was monitored by TEM visualization of purified virus that was treated in a pH range from 3 to 6. The pI of the virus was determined by measurement with the PALS Zeta Potential Analyzer (Brookhaven Instruments).

**CryoTEM and Image Reconstruction.** Frozen-hydrated virus samples were prepared on holey electron microscopy grids as described (17). Briefly, 5  $\mu$ l of purified/concentrated virus sample was applied for 2 min onto a previously glow-discharged copper grid coated with a holey carbon film. The grid was blotted with preheated Whatman no. 2 filter paper to near dryness, flash frozen in a slush of liquid ethane, and transferred into liquid nitrogen. The grid was transferred with a Gatan 626 cryostage (Gatan, Pleasanton, CA) into a Phillips CM120 TEM (Phillips/FEI, Eindhoven, The Netherlands) operated at an accelerating voltage of 100 kV. Focal pairs of electron micrographs were recorded under low-dose conditions (5–10 electrons/ $\text{\AA}^2$ ) at a magnification of  $\times 28,000$ . Micrographs were digitized by using

a Zeiss SCAI scanner (Z/I Imaging, Madison, AL). The step size for scanning was 7  $\mu$ m, corresponding to a pixel size of 2.5  $\text{\AA}$ . Particles were boxed with the autobox subroutine in EMAN (18), and image reconstruction was carried out with SPIDER (19). A total of 718 particles from micrographs recorded at an under-focus of 2.3  $\mu$ m yielded a reconstruction at 34- $\text{\AA}$  resolution. The resolution was subsequently improved to 27  $\text{\AA}$  by using an additional 612 particles recorded at an under focus of 1.5  $\mu$ m. No further contrast transfer focus (CTF) correction was performed, because the resolution was lower than the first zero of the CTF. The resolution was estimated with the Fourier shell correlation method by using a criterion of 0.5 (20).

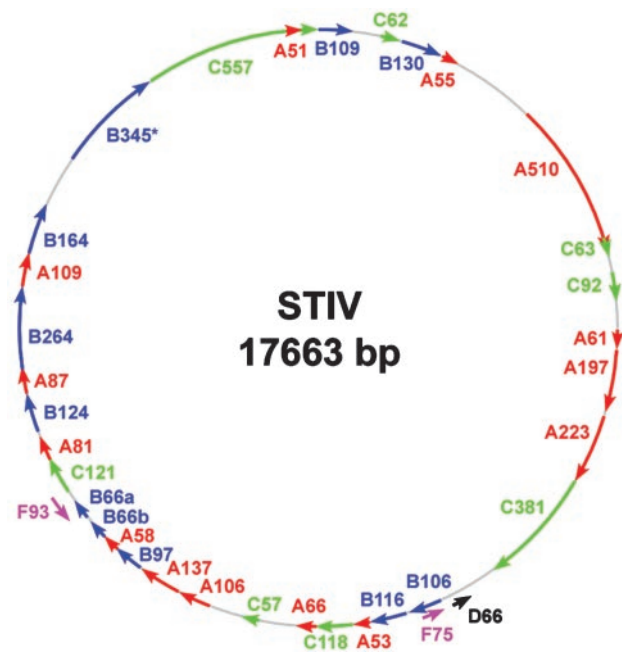
## Results

**Virus Isolation.** Single-colony isolation established a pure virus-producing culture. Phylogenetic analysis of the 16S rRNA gene of isolate YNPRC179 identified the viral host as a close relative of *S. solfataricus*, an aerobic facultative chemolithotroph (15). Under the optimal growth conditions of 80°C, pH 3.3, in DSM88 minimal media, virus particles were observed directly in culture supernatants by TEM analysis. We have no evidence that the virus integrates its viral genome into the host cell chromosome or induces cell lysis. The virus was purified to near homogeneity after sequential filtration of culture supernatants and isopycnic banding on Cs<sub>2</sub>SO<sub>4</sub> gradients. Virus particles banded at 1.236 g/cm<sup>3</sup> in Cs<sub>2</sub>SO<sub>4</sub> and maintained the same morphology as originally observed in the primary enrichment cultures.

Purified virus retained its infectivity as demonstrated by its ability to multiply when introduced into virus-free isolates of *S. solfataricus* strains P1 and P2. The highest amount of virus was observed in *S. solfataricus* P2 after treatment of the purified virus with Proteinase K ( $\approx 50\%$  more virus than control). The purified virus retained infectivity after treatment with 0.3% Triton X-100 (similar to control). Infectivity was lost after treatment with 0.1% SDS or 25% chloroform.

**Identification of the Major Structural Protein.** SDS/PAGE analysis of purified virions revealed an abundant protein with an estimated molecular mass of 37 kDa as well as several minor proteins with estimated masses of 75, 25, 12.5, and 10 kDa. The gene encoding the major 37-kDa virion protein was identified by trypsin digestion of the coat protein from purified virus followed by MALDI mass spectrometry of the product. Five of these fragments matched the theoretical trypsin digests of ORF B345 predicted by ExPASy's PEPTIDE MASS program (Fig. 1). The pI of the virus was determined to be pH 3.26 by dynamic light-scattering  $\zeta$  potential analysis. Other protein bands were not retrieved in sufficient quantities to perform mass spectrometry.

**Genome Analysis.** The virus encapsidates a 17,663-bp circular dsDNA genome (Fig. 1). Like its host genome, the viral genome has a low G+C content (36%) (16). A total of 50 ORFs >50 amino acids can be identified. However, this number of predicted ORFs is reduced to 36 predicted ORFs if one requires each ORF to be >50 amino acids and to be associated with TATA-like elements. These 36 ORFs potentially encode for proteins ranging in size from 5.1 to 57 kDa, and the inferred codon usage of these ORFs shows a codon usage bias similar to that of sequenced *S. solfataricus* (16). A search of the genome for TATA-like elements associated with the Sulfolobales [TTTTTAAA (21)] found three exact matches and 16 other TATA-like elements similar to the Sulfolobales consensus sequence. If the position of these TATA-like elements is considered along with the degree of overlap between downstream ORFs, it suggests that six polycistronic transcripts would encompass 31 of the 36 predicted ORFs. The five remaining ORFs have individual TATA-like elements, suggesting that they may be transcribed separately on monocistronic RNAs. Nearly all of the predicted



**Fig. 1.** Genome map of the 17,663-bp circular dsDNA STIV genome. The 36 predicted ORFs >50 amino acids in length and associated with TATA-like elements are indicated. Reading frames plus 1 (red), 2 (blue), 3 (green), and reading frames minus 1 (black) and minus 3 (purple) are indicated. The major structural protein (B345) identified by mass spectrometry is indicated (\*).

ORF products show no significant similarity to proteins in the public databases. The exceptions to this observation are a region of ORF C557, which is similar to a hypothetical protein of unknown function encoded by *Sulfolobus tokodaii* (22), and ORFs B116 and C92 that show similarity to ORFs of the *Sulfolobus* Rudiviruses, *S. islandicus* rod-shaped viruses (SIRV)1 and SIRV2 (11). Interestingly one of these ORFs, ORF B116, also is homologous to an ORF found in the Lipothrixviruses *S. islandicus* filamentous virus (12) and *Acidianus* filamentous virus

(13). They are not clustered in the genome of any of these viruses.

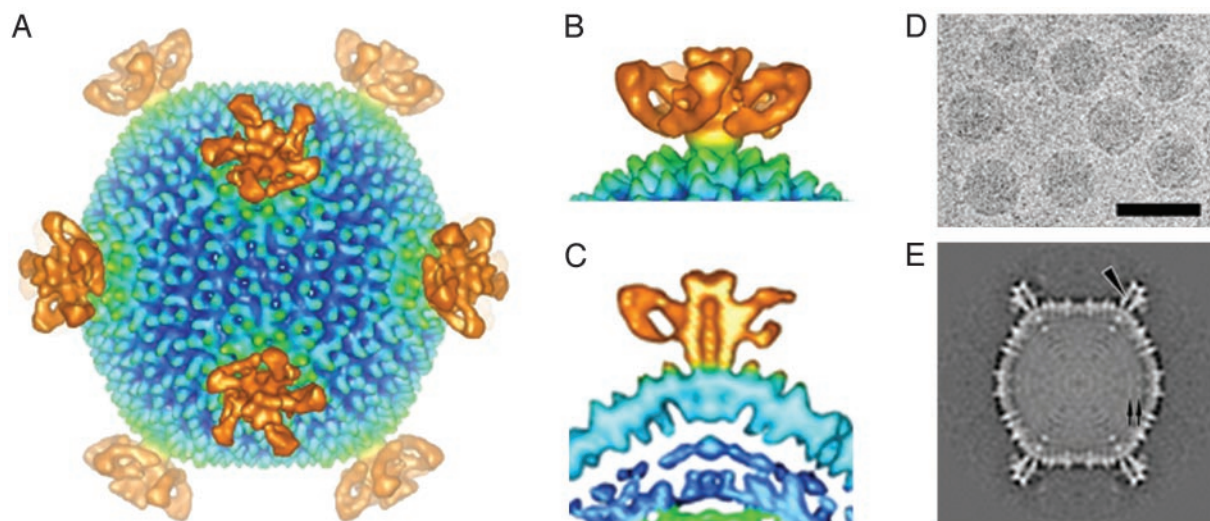
**CryoTEM and Image Reconstruction.** Image reconstruction at  $\approx 27$ -Å resolution revealed a unique virus architecture including a complex structure of appendages at each of the 12 5-fold vertices (Figs. 2 and 3). These appendages are five-sided turret-like structures that have an average diameter of 24 nm and that extend 13 nm above the particle surface. The center of each turret contains an  $\approx 3$ -nm channel that could provide access between the interior and exterior of the virion. There appears to be a density at each end of the channel, indicating that the channel may be blocked and suggesting a possible regulatory role associated with this density.

The structural features of the capsid indicate that the virion is built on a pseudo- $T = 31$  icosahedral lattice, which was previously undescribed (Fig. 3). The diameter of the capsid is  $\approx 74$  nm with a  $\approx 6.4$ -nm shell thickness. The icosahedral asymmetric unit consists of five trimers (pseudo-hexamers) of the major capsid protein plus one additional minor capsid protein at the 5-fold vertex.

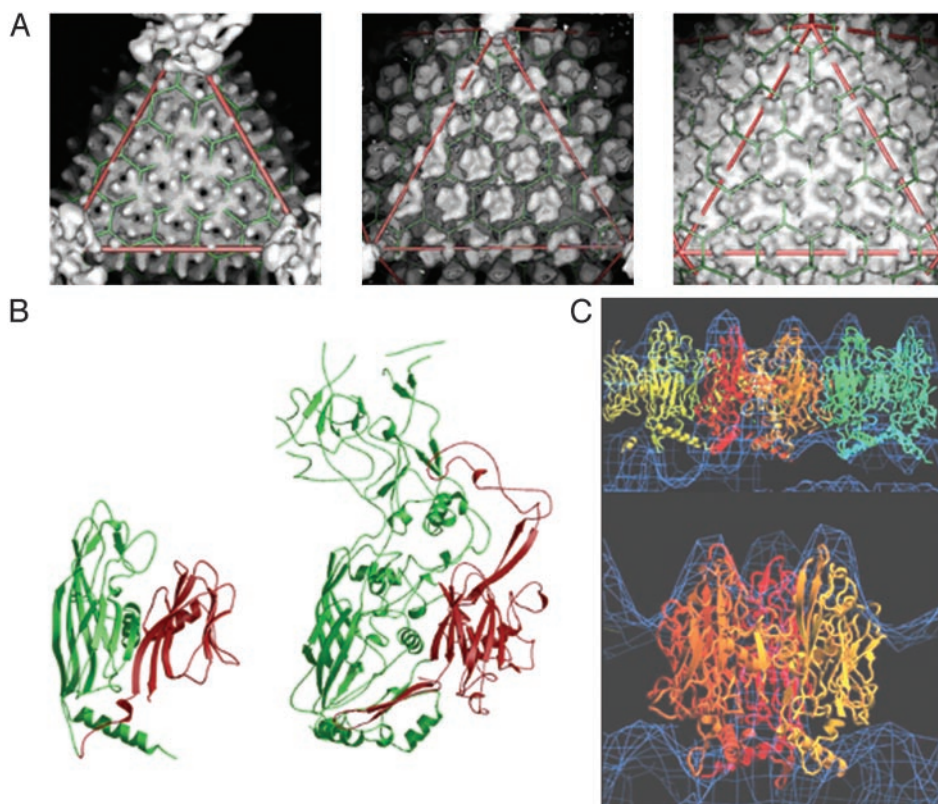
### Discussion

We report here a previously undescribed structural and genetic characterization of an icosahedral virus from a hyperthermoacidophilic Archaeon. The virus was originally isolated from environmental samples taken from hot springs in the Rabbit Creek thermal area located in the Midway Geyser Basin of YNP. The Rabbit Creek springs are characterized by high temperature (70–90°C), acidic pH (2–4), and water geochemistry that is dominated by high levels of sulfate, silicon, iron, sodium, and aluminum (23). We have also detected similar virus particle morphologies from other high-temperature acidic thermal features in YNP (data not shown), suggesting that this virus occurs commonly in YNP.

Like most viruses described to date that replicate in the Crenarchaeota of the domain Archaea, we are unable to detect significant similarity between the potential gene products encoded on the viral genome with those available in public databases. A similar lack of homology to sequences in public



**Fig. 2.** CryoTEM and image reconstruction of STIV. (A) STIV surface features with turret-like projections extending from each of the 5-fold vertices. (B) Side view of turret-like projections extending 13 nm above the surface of the virus surface. (C) Cross section showing a 3-nm central channel in a single turret-like projection. (D) Electron micrograph of vitrified STIV sample recorded at an underfocus of 1.5  $\mu\text{m}$ . (Bar = 1,000 Å.) (E) A 7.5-Å-thick equatorial section of the cryoelectron microscopy map viewed down the icosahedral 2-fold axis. An arrowhead and a pair of arrows indicate the channel in the 5-fold turret and two leaflets of the putative bilayer, respectively.



**Fig. 3.** (A) Surface capsomer architecture of  $T = 31$  STIV (Left), adenovirus (Center), and PRD1 (Right). The icosahedral asymmetric unit (triangle) consists of five trimers (pseudohexamers) of the major capsid protein plus one additional minor capsid protein at the 5-fold vertex. (B) The x-ray crystal structures (ribbon diagrams) of PRD1 (29) (Left) and adenovirus hexon (29) (Right) major coat proteins showing their similar folds. Both contain the “double-jellyroll” motif (red and green). (C) Fitting of the PRD1 P3 coat protein x-ray crystal structure (ribbons) into the STIV cryoimage reconstruction density (blue). Fitting of three trimers corresponding to an icosahedral asymmetric unit of the capsid (Upper). Fitting of a trimer (Lower). The ribbon diagrams of the x-ray atomic structure were ramp-colored from the N terminus of the first (red) to the C terminus of the last trimer (blue).

databases has also been observed in the genome analysis in the sequenced viral genomes of Fuselloviruses *Sulfolobus* spindle-shaped virus (9, 10), Rudiviruses *S. islandicus* rod-shaped virus (11), and Lipothrixviruses *S. islandicus* filamentous virus (12) and *Acidianus* filamentous virus 1 (13) that also replicate in Sulfolobales hosts. This suggests that viruses of the Sulfolobales may have unique processes for carrying out their replication cycles and/or may reflect unique requirements for replication in hyperthermophilic archaeal hosts. Perhaps the ORF conserved in all except the Fuselloviruses is involved in this replication or adaptation to extreme conditions. It is also possible that these viruses can undergo rapid evolution, or that they have been ecologically isolated for a long period due to the unique environments in which they inhabit, which has obscured their relationships at the gene level to other viruses and organisms. It seems probable that a combination of these factors contribute to the genetic diversity of these viruses, but the relative ecological isolation of the Sulfolobales and their viruses may be the most important factor that has resulted in their unique genome composition (24).

The elaborate turret-like structures evident in the reconstruction of the viral capsid are predominant surface features. It is possible that these structures play a role in host recognition and/or attachment. We speculate that the central channel in each turret might act as a portal for translocation of the viral nucleic acid, as is typical of the 5-fold vertices in many viruses (25, 26). Based on these unusual structural features, we propose that this virus be named STIV for *Sulfolobus* turreted icosahedral virus.

The structural organization of the STIV capsid is similar to other viruses with large  $T$  numbers such as human adenovirus (27, 28), bacteriophage PRD1 (29, 30), and the algal virus PBCV-1 (31). The predicted secondary structure of the STIV major capsid protein (see above) is predominately  $\beta$ -sheet. This is similar to the secondary structure of the major capsid proteins of adenovirus, PRD1, and PBCV-1 determined by x-ray crystallography. The atomic structures of these three capsid proteins show a common two- $\beta$ -sandwich fold (Fig. 3). To investigate the relationship between these capsid proteins, the atomic coordinates of the major capsid protein from PRD1 and adenovirus were docked into the 3D map of STIV (Fig. 3). These docking studies suggest that STIV also has an arrangement of two  $\beta$ -sandwich domains that are remarkably similar to those in PRD1 and adenovirus. This is indicated by the characteristic location of the turrets on the outer surface of the major capsid protein with respect to the quasi-equivalent lattice in each case. All three of these viruses also use a pseudo-hexameric structure composed of six  $\beta$ -sandwich domains as the basic building block to fulfill the quasi-equivalent assembly of the capsid. Like PRD1 and PBCV-1, the cryoelectron microscopy map of STIV suggests that it may contain an internal lipid envelope. Biochemical and biophysical analyses are under way to confirm the presence of an inner lipid envelope.

The lack of genomic similarity of these viruses creates the need to look at other similarities between these viruses to infer relationships. An alternative approach of comparison among long-separated viral lineages is the possibility that they share a common innate “self,” which is based on the structural and

assembly principles of the virion. The proposal that molecular organization (folding) of biological macromolecules preceded the appearance of early life forms suggests there may be a conservation of structure (and function) that may be useful for penetrating deep into the history of life (32). The similar organization of the capsid proteins from the bacteriophage PRD1, the eukaryotic adenovirus, and now the archaeal STIV suggests long-range evolutionary relationships among these viruses from all three domains of life.

There are other potential explanations for this astounding similarity. First, there could have been massively convergent evolution (although this is unlikely), because there are many different virus morphologies that can infect Archaea, Eukarya, and Bacteria. A second explanation is that there was horizontal gene transfer among all three domains, a possibility that cannot be ruled out but is also unprecedented and must have happened long ago for all sequence resemblance to have disappeared. Finally, a common ancestor responsible for the innate self still

evident in STIV, PRD1, PBCV-1, and adenovirus is postulated that would have preceded the divergence of the three domains of life >3 billion years ago. The striking similarity in the characteristics of these three major virion proteins of these viruses strongly suggests the latter.

The work reported here further supports the concept that the viral world has lineages that can be traced back to near the root of the universal tree of life (32). Further characterization of STIV and other viruses from hyperthermophilic Archaea is likely to greatly expand our knowledge of evolution of life on earth and provide new insights into the archaeal domain of life.

We thank S. Brumfield and D. Willits for excellent technical assistance. This investigation was supported by grants from the National Science Foundation (MCB 01322156) and the National Aeronautics and Space Administration for support of the Montana State University Center for Life in Extreme Environments Thermal Biology Institute (NAG5-8807) and by support from the National Institutes for Health (Grant GM54076 to J.E.J.).

- Ackermann, H. W. (2001) *Arch. Virol.* **146**, 843–857.
- Rachel, R., Bettstetter, M., Hedlund, B. P., Haring, M., Kessler, A., Stetter, K. O. & Prangishvili, D. (2002) *Arch. Virol.* **147**, 2419–2429.
- Zillig, W., Arnold, H. P., Holz, I., Prangishvili, D., Schweier, A., Stedman, K., She, Q., Phan, H., Garrett, R. & Kristjansson, J. K. (1998) *Extremophiles* **2**, 131–140.
- Rice, G., Stedman, K., Snyder, J., Wiedenheft, B., Willits, D., Brumfield, S., McDermott, T. & Young, M. J. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 13341–13345.
- Stetter, K. (1999) *FEBS Lett.* **452**, 22–25.
- Zillig, W., Kletzin, A., Schleper, C., Holz, I., Janekovic, D., Hain, J., Lanzendorfer, M. & Kristjansson, J. K. (1994) *Syst. Appl. Microbiol.* **16**, 609–628.
- Prangishvili, D., Stedman, K. & Zillig, W. (2001) *Trends Microbiol.* **9**, 39–43.
- Prangishvili, D. (2003) *Res. Microbiol.* **154**, 289–294.
- Palm, P., Schleper, C., Grampp, B., Yeats, S., McWilliam, P., Reiter, W.-D. & Zillig, W. (1991) *Virology* **185**, 242–250.
- Stedman, K. M., She, Q., Phan, H., Arnold, H. P., Holz, I., Garrett, R. A. & Zillig, W. (2003) *Res. Microbiol.* **154**, 295–302.
- Prangishvili, D., Arnold, H. P., Gotz, D., Ziese, U., Holz, I., Kristjansson, J. K. & Zillig, W. (1999) *Genetics* **152**, 1387–1396.
- Arnold, H. P., Zillig, W., Ziese, U., Holz, I., Crosby, M., Utterback, T., Weidmann, J. F., Kristjansson, J. K., Klenk, H. P., Nelson, K. E. & Fraser, C. M. (2000) *Virology* **267**, 252–266.
- Bettstetter, M., Peng, X., Garrett, R. A. & Prangishvili, D. (2003) *Virology* **315**, 68–79.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) (Cold Spring Harbor Lab. Press, Plainview, NY).
- Zillig, W., Stetter, K. O., Wunder, S., Schulz, W., Priess, H. & Scholz, I. (1980) *Arch. Microbiol.* **125**, 259–269.
- She, Q., Singh, R. K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M. J., Chan-Weiher, C. C., Clausen, I. G., Curtis, B. A., De Moors, A., et al. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 7835–7840.
- Dubochet, J., Adrian, M., Chang, J. J., Homo, J. C., Lepault, J., McDowell, A. W. & Schultz, P. (1988) *Q. Rev. Biophys.* **21**, 129–228.
- Ludtke, S. J., Baldwin, P. R. & Chiu, W. (1999) *J. Struct. Biol.* **128**, 82–97.
- Frank, J., Radermacher, M., Penczek, P., Zhu, J., Li, Y., Ladjadj, M. & Leith, A. (1996) *J. Struct. Biol.* **116**, 190–199.
- Van Heel, M. (1987) *Ultramicroscopy* **21**, 111–123.
- Soppa, J. (1999) *Mol. Microbiol.* **31**, 1589–1592.
- Kawarabayasi, Y., Hino, Y., Horikawa, H., Jin-no, K., Takahashi, M., Sekine, M., Baba, S., Ankaï, A., Kosugi, H., Hosoyama, A., et al. (2001) *DNA Res.* **8**, 123–140.
- Braden, H. (2003) Ph.D. dissertation (Montana State University, Bozeman).
- Whitaker, R. J., Grogan, D. W. & Taylor, J. W. (2003) *Science* **301**, 976–978.
- Bothner, B., Dong, X. F., Bibbs, L., Johnson, J. E. & Siuzdak, G. (1998) *J. Biol. Chem.* **273**, 673–676.
- Lewis, J. K., Bothner, B., Smith, T. J. & Siuzdak, G. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 6774–6778.
- Stewart, P. L., Burnett, R. M., Cyrklaff, M. & Fuller, S. D. (1991) *Cell* **67**, 145–154.
- Benson, S. D., Bamford, J. K., Bamford, D. H. & Burnett, R. M. (1999) *Cell* **98**, 825–833.
- Bamford, D. H., Caldentey, J. & Bamford, J. K. (1995) *Adv. Virus Res.* **45**, 281–319.
- Butcher, S. J., Bamford, D. H. & Fuller, S. D. (1995) *EMBO J.* **14**, 6078–6086.
- Nandhagopal, N., Simpson, A. A., Gurnon, J. R., Yan, X., Baker, T. S., Graves, M. V., Van Etten, J. L. & Rossmann, M. G. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 14758–14763.
- Bamford, D. H. (2003) *Res. Microbiol.* **154**, 231–236.