Published in final edited form as:

J Proteome Res. 2012 February 3; 11(2): 1420–1432. doi:10.1021/pr201087v.

Proteomic Analysis of *Sulfolobus solfataricus* During *Sulfolobus* Turreted Icosahedral Virus Infection

Walid S. Maaty¹, Kyla Selvig¹, Stephanie Ryder¹, Pavel Tarlykov¹, Jonathan K. Hilmer¹, Joshua Heinemann¹, Joseph Steffens¹, Jamie C. Snyder², Alice C. Ortmann^{2,3}, Navid Movahed¹, Kevin Spicka¹, Lakshindra Chetia¹, Paul A. Grieco¹, Edward A. Dratz¹, Trevor Douglas¹, Mark J. Young², and Brian Bothner^{1,*}

¹Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT

²Departments of Microbiology and Plant Sciences, Montana State University, Bozeman, MT

³Department of Marine Science, University of South Alabama, Mobile, AL

Abstract

Where there is life, there are viruses. The impact of viruses on evolution, global nutrient cycling, and disease has driven research on their cellular and molecular biology. Knowledge exists for a wide range of viruses, however, a major exception are viruses with archaeal hosts. Archaeal virus-host systems are of great interest because they have similarities to both eukaryotic and bacterial systems and often live in extreme environments. Here we report the first proteomics-based experiments on archaeal host response to viral infection. *Sulfolobus* Turreted Icosahedral Virus (STIV) infection of *Sulfolobus solfataricus* P2 was studied using 1D and 2D differential gel electrophoresis (DIGE) to measure abundance and redox changes. Cysteine reactivity was measured using novel fluorescent zwitterionic chemical probes that, together with abundance changes, suggest that virus and host are both vying for control of redox status in the cells. Proteins from nearly 50% of the predicted viral open reading frames were found along with a new STIV protein with a homolog in STIV2. This study provides insight to features of viral replication novel to the archaea, makes strong connections to well described mechanisms used by eukaryotic viruses such as ESCRT-III mediated transport, and emphasizes the complementary nature of different omics approaches.

Keywords

Archaea; virus infection; *Sulfolobus solfataricus* strain P2; STIV; *Sulfolobus* turreted icosahedral virus; Proteomics; virus–host interaction; LC/MS/MS; liquid chromatography mass spectrometry; differential gene expression; membrane protein; VAPs; Virus-associated pyramids; 2-D Fluorescence Difference Gel Electrophoresis; Thiol-reactive maleimide probe

Introduction

The relatively recent demarcation of archaea as a third domain of life and the exotic viruses associated with these organisms are currently very active research topics.^{1–5} Our understanding of archaeal viruses and how they interact with their hosts lags well behind viruses associated with bacterial and eukaryotic hosts and this is especially true of viruses

*Corresponding Author: Dr. Brian Bothner; bbothner@chemistry.montana.edu; 111 Chemistry and Biochemistry Bldg, Chemistry and Biochemistry Department, Montana State University, Bozeman, MT 59715, Phone: (406) 994-5270, Fax: (406) 994-5407.

Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

that infect members of the Crenarchaea. The basic viral replication cycle is just beginning to be understood, even for the best described viruses that infect *Sulfolobus* spp., such as *Sulfolobus* spindle-shaped virus (SSV)⁶ and *Sulfolobus islandicus* rod-shaped virus (SIRV).⁷ What is clear at this point is that studies of archaea are bringing insight to evolution of the domains of life and exciting new biology such as virus-associated pyramids (VAPs) on infected cell surfaces and viruses that change morphology after release.^{8–10}

STIV was originally isolated from enrichment cultures of a high temperature (~80°C) acidic (~pH 3) hot spring in Yellowstone National Park (YNP).¹¹ It was the first icosahedral virus described from the archaeal domain of life. It can infect *S. solfataricus* (P2), originally isolated in Italy, as well as *Sulfolobus* species found in YNP. Structural models based on cryo-electron microscopy and image reconstruction revealed a capsid with pseudo T=31 symmetry, turret structures at each of the five-fold axes, and an internal lipid layer.¹² Surprisingly, STIV has a clear common ancestry at the structural level with both prokaryotic and eukaryotic viruses.¹³ Subsequent analysis determined that the capsid is composed of nine viral proteins and an internal layer of cyclic tetraether lipids.¹⁴ The 17.6 kb double stranded DNA genome has 37 open reading frames that code for proteins that, for the most part, lack homologs at the sequence level. Structural models based on X-ray diffraction are now available for 4 proteins including the major capsid protein.^{13, 15–17}

A majority of the viruses with archaeal hosts are believed to be non-lytic.^{18–21} However, recent evidence that STIV is lytic suggests that this topic may need to be revisited.²² The basics of the STIV replication cycle and a transcriptome analysis of the host response to infection have been reported²², providing the first glimpse of an archaeal response to viral infection. The prior transcriptome analysis and the data presented herein are from the same sample set of a near synchronous infection of > 95% of *S. solfataricus* strain (SsP2-2-12). Transcription of STIV genes was evident 8 hours post infection (hpi) and peaked at 24 hpi, with little temporal variation of viral gene transcription. The microarray analysis of infection detected changes in expression for 177 host genes (~ 5%), with 124 up-regulated and 53 down-regulated. The up-regulated genes were primarily involved with DNA replication and repair or of unknown function, while the down-regulated genes were associated with energy production and metabolism. A surprising discovery was made from a time-course study of cells after STIV infection when dramatic pyramids appeared on the cell surface beginning at 32 hpi.⁸ Soon after, a second report of viral associated pyramids (VAPs) on the closely related *S. islandicus* infected by SIRV1 was published.⁹ An investigation of how infection is manifest at the protein level has yet to be reported on any of these systems.

The central objective of this study was to extend our understanding of the STIV-*Sulfolobus* viral-host interaction beyond the level of transcriptional regulation, using proteomics and activity-based protein profiling. To accomplish this, a time course analysis of the proteins that change in STIV infected *S. solfataricus* was performed using 1D and 2D-DIGE, followed by protein identification using mass spectrometry. Viral proteins were detected at 24 hpi and peaked at 32 hpi. Gel image analysis of protein abundance revealed nine host proteins that increased and one that decreased over the course of infection. This data was augmented with the first use of activity-based protein profiling in archaea. Viral proteins were found in both cytoplasmic and membrane fractions. No co-regulated changes at the protein and mRNA levels were found, a result that has been reported for other systems²³, however, the identified cellular networks were the same. Additionally, whereas nearly half of the regulated genes were of unknown function, changes in the proteome highlight known proteins with strong connections to eukaryotic processes such as cell division and vesicular transport.

Materials and Methods

Virus purification

STIV production and purification was carried out by growing the *S. solfataricus* strain P2-2-12 in DSMZ Medium 182 (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium182.pdf) at pH 2.5 as previously described.²²

Viral infection—Batch cultures of three independent pairs of *S. solfataricus* strain P2-2-12 cultures were infected with STIV at a multiplicity of infection (MOI) of ~1.5 to 2 and sampled over time as previously described²². Samples were removed from the cultures and processed in parallel for mRNA and protein assays. Cells used for proteomics were pelleted, resuspended in 0.5 ml of sterile PBS, flash-frozen in liquid nitrogen, and stored at –80°C until analysis. Microarray hybridizations and analysis were conducted as described in details by Ortman et al.²²

2-D DIGE

Sample extracts were prepared from cell suspensions washed with PBS. Cells were lysed with a combination of freeze-thaw and lysis buffer (30 mM Tris-HCl pH 8.5 (4°C), 7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 0.5% IPG carrier ampholytes and a cocktail of protease inhibitors) for 60 min. and the supernatant was clarified by centrifugation. Proteins were purified and concentrated by precipitation with a 5-fold volume of cold acetone, and re-suspended for 1 hr in lysis buffer without DTT. Protein concentration was measured with the RC/DC Protein Assay Kit (Bio-Rad). Samples were kept frozen until use. Protein samples were labeled with CyDyes using the minimal labeling method according to the manufacturer's protocol (GE Healthcare, [http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/EF8087141F244296C1257628001D0763/\\$file/18_1164_84_AB.pdf](http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/EF8087141F244296C1257628001D0763/$file/18_1164_84_AB.pdf)). Approximately 1 out of 100 lysine side chains are labeled. Briefly, 50 µg of each protein extract was labeled separately at 0°C in the dark for 30 min with 400 pmoles of the N-hydroxysuccinimide esters of cyanine dyes (Cy3 and Cy5 CyDyes;) dissolved in 99.8% DMF (Sigma). The internal standard, an equimolecular mixture of all the protein extracts, was labeled with Cy2. Labeling reactions were quenched by the addition of 1 µL of a 10-mM L-lysine solution (Sigma) and left on ice for 10 min. Cy2, Cy3 and Cy5 labeled samples were combined appropriately and mixed with rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS) containing 50 mM DTT and 0.5 % IPG.

2-DE was performed as described elsewhere²⁴, using precasted IPG strips (pH 3–11 NL, non-linear, 24 cm length; GE Healthcare) in the first dimension (IEF). Labeled samples were combined with a maximum of 450 µl rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 0.5 % IPG buffer pH 3–11 NL, 40 mM DTT, and a trace of bromophenol blue) and loaded onto IPG strips. Typically, 150 µg proteins were loaded on each IPG strip and IEF was carried out with the IPGPhor II (GE Healthcare). Focusing was carried out at 20°C, with a maximum of 50 µA/strip. Active rehydration was achieved by applying 50 V for 12 h. This was followed by a stepwise progression of 500 V for 500 Vhr, gradient ramp from 500 to 1000 V for 1 h, gradient ramp from 1000 to 3000 V for 1 h, gradient ramp from 3000 to 5000 V for 1 h, gradient ramp from 5000 to 8000 V for 1 h, gradient ramp to 8000 V over 1 h, then 8000 V constant for a total of 44,000 Vhr. After IEF separation, the strips were equilibrated twice for 15 min with 50 mM Tris-HCl, pH 8.8, 6 M Urea, 30% glycerol, 2% SDS and a trace of bromophenol blue. The first equilibration solution contained 65 mM DTT, and 153 mM iodoacetamide was added in the second equilibration step instead of DTT. The strips were sealed on the top of the gels using a sealing solution (0.75% agarose in

SDS-Tris HCl buffer). The second-dimension SDS-PAGE was performed in a Dalt II (GE Healthcare), using 1 mm-thick, 24-cm, 13% polyacrylamide gels, and electrophoresis was carried out at a constant current (45 min at 2 W/gel, then at 1 W/gel for ~16h at 25°C). When relevant, gels were stained with SYPRO[®] Ruby or Coomassie[®] Brilliant Blue stains for spot picking.

Image acquisition and analysis

Gels were scanned using a Typhoon Trio Imager according to the manufacturer's protocol (GE Healthcare) at 100 μ m resolution. Images were subjected to automated difference in gel analysis using Progenesis SameSpots software version 3.0.2 (Nonlinear Dynamics Ltd.). The Cy3 gel images were scanned at an excitation wavelength of 532 nm with a long pass emission wavelength of 570 nm, Cy5 gel images were scanned at an excitation wavelength of 633 with a long pass emission wavelength of 670 nm, while the Cy2 gel images were scanned at an excitation wavelength of 488 nm with 520 nm emission filter. Gel spots were co-detected as DIGE image pairs, which were linked to the corresponding in-gel Cy2 standard. Between gel comparisons were performed utilizing the Cy2 in-gel standard from each image pair, using Progenesis SameSpots. The gels are then stored in 1% acetic acid at 4°C until spot excision.

To check for protein specific bias in Cydye labeling, a separate analysis in which uninfected SsP2 lysate was separately labeled with all three Cy-dyes was performed. Labeled samples were mixed and 2D-DIGE was performed. Five *S. solfataricus* proteins with a bias toward Cy-dye 3 which could result in a false positive using a 1.5 fold-cutoff were found. None of these proteins were among the differentially regulated protein spots at the higher fold cut-off used in this study; therefore, dye swapping in the DIGE analysis was not used.

Membrane preparation

The membrane proteins from *S. solfataricus* 2-2-12 were prepared using a cell fractionation and ultra-centrifugation protocol modified from Lower et al.²⁵ *S. solfataricus* was grown to OD₆₅₀ ~0.3 and cells were harvested at $3,944 \times g$ for 10 minutes. The cell pellet was washed with PBS pH (7.4) and resuspended in 5 ml of 25 mM phosphate buffer (PB) (pH 7.4) containing 25 μ l of a 7 $\times$ Complete protease inhibitor cocktail (Roche), and 50 μ l of DNase I/RNase. The cells were lysed by freezing and thawing in liquid N₂ followed by probe sonication at 30% duty cycle for 1 min. (repeated 3 times) on ice. The lysate was centrifuged at $1,000 \times g$ for 10 min at 4°C to remove debris. Supernatant liquid was centrifuged at $100,000 \times g$ (30,000 RPM) for 75 min at 4°C to separate the membrane fraction from soluble proteins. The membrane containing pellet was then washed by resuspension in 5 ml of 20 mM sodium acetate (pH 5.0) containing 0.5 M NaCl and the $100,000 \times g$ centrifugation step was repeated. The pellet was resuspended in 5 ml of 25 mM PBS containing 0.4% Triton X-100 and 125 mM NaCl. Membrane material was then pelleted with a third centrifugation leading to a final pellet which was resuspended in 25 mM PBS and an equal volume of 4 $\times$ SDS-PAGE gel-loading buffer. Samples were boiled prior to separation on 4–20% SDS-PAGE.

Zdye maleimide probe for thiol labeling

S. solfataricus proteins were labeled with a custom made fluorescent Zdye (ZB-M LC-01-56) coupled to a maleimide (Figure 2) [Dratz, E. A. and P. A. Grieco. Novel Zwitterionic Fluorescent Dyes for Labeling in Proteomic and Other Biological Analyses. USA patent Number 7,582,260, 9/1/2009, Montana State University, Dratz, E. A. and P. A. Grieco. Novel Zwitterionic Fluorescent Dyes for Labeling in Proteomic and Other Biological Analyses. USA patent Number 7,833,799, Montana State University, 11/16/2010.]. A publication detailing synthesis and properties of the dye is forth coming.

The labeling reactions were performed on 3 biological replicates using dye at a final concentration of 5 μ M in PBS, pH 7.4, for 20 min at room temperature. Reactions were quenched with 2D gel-loading buffer containing 40 mM DTT, loaded onto 24 cm, 3-11NL isoelectric focusing gel strips. 2D gels were scanned on the Typhoon fluorescence imager using a standard 488/510 nm filter set. Gels were then stained with SYPRO[®]Ruby (Molecular Probes[™]) to confirm equal protein loading before DIGE analysis. Finally, the gels were stained with Coomassie GelCode[™] Blue Safe (Thermo Scientific) Protein Stain for visible spot picking.

***In vitro* analysis of C92**

C92 (Δ 1–27) was cloned in-frame with N-terminal 6His-tag into the pDEST14 expression vector (Gateway system, Invitrogen), according to the manufacturer's protocol. *Escherichia coli* BL21 (DE3) PlysS strain (Invitrogen) was used for the protein expression. Protein was purified using Ni-NTA affinity gravity flow column (Novagen). Determination of C92 molecular mass in solution was carried out by size exclusion chromatography using an ÄKTApurifier[™] FPLC (GE Healthcare) with a BioSep_SEC_S2000 (Phenomenex[®]) column, equilibrated with PBS (pH 7.4) that had been calibrated using the molecular mass standards Vitamin B12 (1,350 Da), Myoglobin (17,000 Da), Ovalbumin (44,000 Da), γ -Globulin monomer (158,000 Da), γ -Globulin dimer (316,000 Da) and Tyroglobulin (670,000 Da).

Protein identification

Protein spots of interest were excised from the gels, washed, in-gel reduced and S-alkylated, followed by digestion with porcine trypsin (Promega) overnight at 37°C^{14, 24}. The solution containing peptides released during in-gel digestion were transferred to sample analysis tubes for mass analysis. LC/MS/MS used an integrated Agilent 1100 liquid chromatography–mass-selective detection (LC-MSD) trap (XCT-Ultra 6330) controlled with ChemStation LC 3D (Rev A.10.02). The Agilent XCT-Ultra ion trap mass spectrometer is fitted with an Agilent 1100 CapLC and Chip Cube under the control of MSD trap control version 5.2 Build no. 63.8 (Bruker Daltonics GmbH). Injected samples were first trapped and desalted on the Zorbax 300SB-C18 Agilent HPLC-Chip enrichment column (40 nl volume) for 3 min with 0.1% formic acid delivered by the auxiliary pump at 4 μ l/min. The peptides were reverse eluted and loaded onto the analytical capillary column (43 mm $\times$ 75 μ m ID, also packed with 5 μ m Zorbax 300SB-C18 particles) and connected in-line to the mass spectrometer using the ChipLC ESI spray needle, with a flow of 600 nl/min. Peptides were eluted with a 5 to 90% acetonitrile gradient over 16 min. Data-dependent acquisition of collision induced dissociation tandem mass spectrometry (MS/MS) was utilized. Parent ion scans covered the m/z range 400 to 2,200 at 24,300 m/z -s. MGF compound list files were used to query an in-house SsP2 database using MS and MS/MS ion mass tolerances of 1.2 and 0.5 amu respectively. Positive identification required two significant peptides based on MASCOT (Matrix science, London, UK) MOWSE scores >32 ($p < 0.05$) and Phenix GeneBio z-Score of >8. Protein fold recognition using 1D and 3D sequence profiles, coupled with secondary structure and solvation potential were performed using DUF²⁶ and PHYRE (Protein Homology/analogy Recognition Engine; <http://www.sbg.bio.ic.ac.uk/phyre/index.cgi>)²⁷. The STIV genome map was constructed using Vector NTI Advance 11.0 (Invitrogen). Multiple sequences alignments were performed using CLUSTAL 2.1.

Results and Discussion

To add depth to our understanding of STIV replication and the biology of Archaea, a parallel transcriptomic and proteomic analysis of *S. solfataricus* strain SP2-2-12 during

STIV infection was conducted. Global transcriptome analysis showed that viral mRNA expression rose rapidly after 8 and peaked at 24 hpi, see Figure S1-A. Viral gene expression during infection of SP2 ATCC stock strain and the clonal isolate SP2-2-12 are very similar, except for a delay in the timing of gene expression and viral release in the SP2 ATCC strain, as shown in Figure S1. Two other crenarchaeal viruses, STIV2 and SIRV1, were recently reported to also have similar narrow host selectivity^{9, 28}. Together with our results, this suggests that previous reports that crenarchaeal viruses are predominantly non-lytic should be reinvestigated.

Changes in the proteome

Based on the microarray expression results for *Sulfolobus* SP2-2-12, parallel samples at 24 and 32 hpi were selected for proteomic analysis. Total soluble protein from control and infected cell lysates was labeled with DIGE dyes, combined and analyzed using standard 2D-DIGE methodology.²⁹ Greater than 900 spots were detected on each gel (Figure 1) using Progenesis SameSpots analysis.³⁰ After filtering to remove spot irregularities, 807 protein spots were used in the analysis across all 9 gels, which is consistent with our previous 2D DIGE experiments.^{29, 31} Using a 1.8 fold change cutoff, there were 11 regulated protein spots at 24 hpi and 29 at 32 hpi. Protein spots were identified using in-gel proteolysis and LCMS/MS analysis²⁴ followed by searching of a database containing all ORFs in *S. solfataricus* and STIV greater than 50 amino acids using Phenix and MASCOT search algorithms. Previous experience with STIV indicated that non-annotated reading frames are used¹⁴, therefore, this larger database was adopted to minimize false negatives in the preliminary IDs. A minimum of two peptides with significant scores were the basis for protein identification. Analysis of 20 regulated protein spots led to the identification of 10 host and 9 STIV encoded proteins (Tables 1 & 3). For the host proteins, 9 increased in abundance while 1 decreased (Table 1).

The limited number of host proteins showing statistically significant changes in abundance was initially surprising, considering the synchronous timing of infection and the wide scale changes observed in mRNA²². Because differential expression is based on spots that show statistically significant changes ($p=0.05$ $q=0.1$) for a given fold change, highly variable spot intensity between biological replicates could reduce the number of spots that showed a significant difference. However, the calculated standard deviation as a percentage of spot intensity between biological replicates was, on average, less than 20% of spot volume, indicating that variability in spot intensity between biological replicates was not a factor in the differential analysis of protein expression (Figure S2).

The host protein showing the greatest change in abundance was SSO0209, a putative N-acetyltransferase. N-acetyltransferases constitute a superfamily of functionally diverse enzymes that catalyze the transfer of an acetyl group from acetyl-Coenzyme A to the primary amine of a wide range of acceptor substrates. The use of histone acetylation to regulate gene transcription in eukaryotes is well known^{32, 33} and a number of viruses, including HIV and HPV, regulate N-acetyltransferases.^{34–38} Global changes in protein acetylation have been associated with metabolic regulation^{39, 40} which is consistent with the changes in the transcriptomics data.²²

Cell division and intracellular transport proteins

Manipulation of host cell machinery involved with cell division is a hallmark of nearly all viruses. The increase in abundance of two proteins that are members of the recently described cell division family of proteins, CdvA and CdvB, SSO0911 and SSO0881 provides the first evidence at the protein level for involvement of Cdv proteins in archaeal viral replication. A three-gene operon composed of CdvA, B, and C has been found in most

crenarchaea^{41–43} and comprises the minimal machinery needed for cell division. CdvA and CdvB form co-localized oligomers segregating chromosomes and are associated with the leading edge of constriction during cytokinesis.⁴² CdvC encodes an AAA+ ATPase believed to be involved with disassembly of the CdvA and CdvB complexes. CdvB and CdvC are similar to type E endosomal sorting proteins of eukaryotes that form the ESCRT-III sorting complex. CdvB is a positively charged coiled-coil protein and all crenarchaea that have Cdv genes have multiple copies of CdvB-like proteins.⁴² The third member of the operon, CdvA, appears to be unique to archaea. Structural prediction suggests that CdvA is a coiled-coil protein possibly similar to myosin, tropomyosin, or cingulin-like proteins. Such a structure would be consistent with a role for CdvA proteins in ESCRT-like daughter chromosome sorting processes or possibly as part of the yet to be delineated archaeal cytoskeleton.

The *S. solfataricus* cdv operon of genes SSO0911, 0910, and 0909 (CdvA, B, and C respectively) were all upregulated at the mRNA level during STIV infection.²² At the protein level, CdvA (SSO0911) was the only member of this Cdv operon that increased in abundance. This could be because SSO0910 and SSO0909 were below the level of detection in the proteomics experiment. Beyond the obvious biological significance of understanding the role of cell division proteins in viral infection, the ESCRT-III family is of particular interest because they are involved in eukaryotic protein sorting, endosomal vesicle formation, and budding of enveloped viruses^{44, 45} *S. solfataricus* ESCRT proteins have been found in secreted vesicles⁴⁶ and in purified viral samples.¹⁴ SSO0881 is one of four CdvB homologs in *S. solfataricus* (the other genes are SSO0451, SSO0619, and SSO0910).

The CdvB ESCRT-III like proteins of eukaryotes have microtubule interacting and transport (MIT) domains at the C-terminus. This domain is missing from the archaeal homologs, and instead has been reported to be replaced by a helix-turn-helix motif that may be used for DNA binding.⁴² Secondary structural prediction of SSO0881 was consistent with this idea (Figure S3) and the ability to bind DNA is consistent with a role in cell division and segregation of polynucleotides. The specific cellular role of SSO0881 is currently unknown, but, it was one of only two host proteins that co-purify with STIV virus particles.¹⁴ Recent work also puts this protein in the Snf7 protein family that is part of the ESCRT complex which in eukaryotes manages protein sorting and transport from the endosome to the vacuole/lysosome.

Viruses known to use the ESCRT pathway include many retroviruses, such as human immunodeficiency virus (HIV)^{47, 48} and murine leukemia virus (MLV)⁴⁷, as well as viruses from other families, such as vesicular stomatitis virus⁴⁹ and herpes simplex virus (HSV)⁵⁰. Disruption of ESCRT function or mutation of viral late domains generally prevents the separation of virions from the cell membrane. Structural prediction of the hypothetical protein SSO2632, which was also regulated, using Phyre 3D revealed a strong similarity to myosin/tropomyosine like proteins, which also play crucial roles in eukaryotic cell division and transport, further strengthening this idea. The finding of ESCRT III and myosin-like proteins among the regulated proteins and mRNA, suggests that STIV modulates the ESCRT system for transport or release. The role of ESCRT proteins in viral transport and how this could relate to STIV and other archaeal viruses has been covered in a recent review.⁵¹

Fibrillarin-like pre-rRNA processing protein, SSO0940, was another regulated protein with strong ties to viral replication in eukaryotes. Fibrillarin is a small nucleolar RNA-processing protein that catalyzes methyl transfer reactions⁵². HIV Tat protein specifically co-localizes with fibrillarin in the nucleoli of *Drosophila* oocyte nurse cells. Tat expression is accompanied by a significant decrease of cytoplasmic ribosomes, which is apparently related to an impairment of ribosomal rRNA precursor processing.⁵³ Other viral proteins

such as porcine arterivirus nucleocapsid protein⁵⁴ and non-structural protein 3b, a protein specifically encoded by the severe acute respiratory syndrome coronavirus (SARS-CoV)⁵⁵ associate with fibrillarin. A suggested outcome of these interactions is the modulation of host cell function through rRNA precursor processing and control of ribosome biogenesis. Other viruses such as, herpes simplex virus 1⁵⁶ and groundnut rosette virus, use fibrillarin to target viral proteins to the nucleolus for assembly of viral ribonucleoprotein particles^{57, 58}. Because *Sulfolobus* lacks a nucleus, we speculate that SSO0940 is involved in viral replication via rRNA processing/ribosome assembly through specific interactions with one of the STIV proteins.

Oxidative stress during infection

Disulfide Oxidoreductase SsPDO (SSO0192) is up regulated in the STIV infected cells. SSO0192 was reported to be involved in the reduction of both the Bcp1 and Bcp4 proteins⁵⁹ and is a substrate of thioredoxin reductase SSO2416 in *S. solfataricus*⁶⁰. SSO0192 belongs to the Thioredoxin reductase (TRX)-Glutaredoxin reductase (GRX)-like family of proteins that, among other functions, have a central role in the formation and maintenance of cytoplasmic disulfide bonds.⁶¹ Interestingly, Thioredoxin reductase-1 (TR1) negatively regulates the activity of the HIV-1 encoded transcriptional activator, Tat, in human macrophages.⁶² It was found that Tat-dependent transcription and HIV-1 replication were significantly increased in human macrophages when TR1 activity was reduced and the effect was independent of the redox-sensitive transcription factor, NF- κ B. This suggests that upregulation of disulfide oxidoreductase is an antiviral mechanism employed by archaea as well as eukaryotic cells.

Peroxiredoxin (SSO2613), also known as Bcp4, is a general cellular stress response protein and was the only identified host protein that decreased in abundance during STIV infection. Bcp-4 is a substrate of the disulfide oxidoreductase discussed above, and plays an important role in the peroxide-scavenging system in *S. solfataricus*^{60, 63, 64}. Peroxiredoxin homologs are prevalent in thermophiles and *S. solfataricus* has four orthologs: Bcp1 (SSO2071), Bcp2 (SSO2121), Bcp3 (SSO225) and Bcp4 (SSO2613).⁶⁵ It has been proposed that the Bcps are part of an antioxidant system using Bcp1 and Bcp4 to prevent endogenous peroxide accumulation, while Bcp2 and Bcp3 are induced in response to external peroxides.⁶⁰ Recently our group showed that Bcp2 was significantly up regulated in response to H₂O₂ oxidative stress.²⁹ STIV apparently counteracts to some extent, host upregulation of SSO0192 by down regulating SSO2613, which is a downstream target of SSO0192. This would be analogous to the increase in HIV replication with TR1 down regulation.⁶² In addition, proteomic analysis of swine fever virus⁶⁶ and Respiratory Syncytial Virus (RSV) infection⁶⁷, confirm changes in peroxiredoxin (Prdx-1, -3, and -4) oligomer status and total abundance during infection. From a cellular standpoint, Prdx-1 and -4 are essential for preventing RSV-induced oxidative damage to a subset of nuclear intermediate filament and actin binding proteins in epithelial cells. Retroviruses may counter such effects, as it was recently shown that peroxiredoxin-1 expression was decreased in H4IIE cells in the presence of a retroviral vector.⁶⁸ Therefore, this archaeal virus system is behaving very similarly to well studied human viruses with regard to infection induced changes in oxidative stress.

Changes in cellular redox state

If the host and virus are vying for control over redox sensitive systems in the cell, we reasoned that it might be possible to find specific proteins that are affected. Reactive cysteine residues in proteins are critical components in redox signaling, protein folding, and can be used as reporters of cellular redox state. Using a thiol reactive maleimide probe, we monitored protein redox state across the entire proteome. Changes in the redox state of proteins were visualized by coupling a reactive group to a novel zwitterionic fluorescent tag.

The ZB-maleimide (ZBM) probe (Figure 2) was designed to overcome limitations of other cysteine labeling reagents on 2D gels.⁶⁹

Total protein from control and infected cells at 24 and 32 hrs was reacted with the probe, and then analyzed using 2D DIGE. More than 300 fluorescent spots were visible across replicate gels (Figure 2). For reference, there are 1863 proteins with at least one cysteine in SsP2 (see Figure 2D and Supplemental Figure S4). Ten protein spots exhibited significant differential labeling in response to viral infection, with 4 increasing and 6 decreasing in label. Proteins were identified from six of the spots (Table 2). Two of the identified proteins had increased labeling during STIV infection (SSO2044, Glutamate dehydrogenase; SSO0421, Transitional endoplasmic reticulum ATPase) and 4 were less reactive (SSO0564, ATP synthase subunit B; SSO0282, Thermosome beta subunit; SSO1151, Conserved hypothetical putative tldD protein; SSO1134, Heterodisulfide reductase subunit C). Protein identification from the remaining four spots was unsuccessful due to insufficient quantities.

SSO0421 was present in spots 13 and 18 suggesting that the protein was posttranslationally modified. Post-translational modifications (PTMs) are a common and highly rapid way in which protein activity can be regulated. Based on gene annotation and functional characterization, the presence of enzymes involved with the addition and removal of PTMs such as methylation, acetylation, phosphorylation, glycosylation, and N-terminal processing are present in *S. solfataricus* (P2)^{29, 65, 70, 71}. Finding the targets and frequency of PTM is a challenging task and currently, little is known about the use of PTM in archaea. The ability to detect PTMs at the level of the proteome, without limiting the scope of the experiment, is a strength of the 2D-DIGE approach. Changes in protein pI and/or MW are caused by most PTMs leading to altered gel mobility and changes in spot intensities. Proteins with less than complete modification at a specific site will be found in more than one spot and the ratios can be used to follow modification kinetics.

Glutamate dehydrogenase labeling increased significantly upon infection, indicating greater accessibility of reduced thiols or reduction of previously oxidized cysteine side chains. Importantly, based on DIGE experiments this protein did not change in abundance, so the change detected reports on regulation at the level of activity, independent of abundance. Glutamate dehydrogenase (SSO2044) has only one cysteine residue. Surprisingly, this cysteine is not only conserved in the 4 annotated glutamate dehydrogenases in SP2 (gdhA-1, gdhA-2, gdhA-3, gdhA-4) but also in the human homologues (hGDH1 and 2). This enzyme plays a crucial role in amino acid metabolism and transport and is involved with Human Cytomegalovirus (HCMV) infection by increasing the production of glutamine.⁷² An increase in glutamine in other systems has been revealed by metabolic flux studies^{73, 74} and cells starved of glutamine failed to produce infectious virions. Also, glutamate dehydrogenase activation can help maintain the cellular energy balance for viral specific lipid production.⁷² This link is particularly interesting because, as we have shown, the internal membrane of STIV is composed of a subset of host lipids.¹⁴

Heterodisulfide reductase subunit C (hdrC-2, SSO1134) had a much lower level of labeling in the virally infected cells (Figure 2 and Table 2). It contains 8 cysteine residues which are grouped into 2 sequence motifs characteristic of Fe₄S₄ clusters as in ferredoxin iron-sulfur binding proteins. This active-site cluster is directly involved in mediating heterodisulfide reduction⁷⁵ and catalyzes the formation of coenzyme M (CoM-SH) and coenzyme B (CoB-SH) by the reversible reduction of the heterodisulfide, CoM-S-S-CoB.⁷⁶ Decreased labeling of SSO1134 could indicate that more of the protein is in the holo form providing the cells with increased control over disulfide formation. The high levels of disulfide bonds in intracellular proteins in hyperthermophiles and their viruses^{16, 77}, make this a particularly interesting result.

Viral Protein Expression

In addition to changes in the host proteome after STIV infection, 13 STIV proteins were identified. Nine viral encoded proteins were identified from 2D gel spots that increased in abundance. These were assigned to the open reading frames A223, B109, B116, B129, B130, B264, B345, C381, and C92. Four of these (B345, B109, B130, and C92) were found in more than one horizontally displaced spot (Figure 1, Table 3), indicating the presence of post-translational modifications of these proteins that create protein isoforms. A previous analysis of purified STIV particles had a similar pattern of spots for B345, the major capsid protein.¹⁴ The majority of the 37 STIV ORFs have no close sequence homolog in other known genomes and of those identified by 2D-DIGE, A510, A55, A78, B109, B116, and B130 currently have no assigned function. It is known that A55, A78, B109, B130, A223, B345 and C381 are present in STIV particles¹⁴, explaining the high levels of expression that we found. Previously it was found that C381 and A223 have sequence similarity to PRD1 P5 vertex proteins and together fit nicely into the electron density observed for the STIV virus turret.¹⁴ Prior sequence analysis and structural prediction indicated that B164 is similar to the Poxvirus ATPase family.¹⁴ Structure predication using PHYRE showed that B264 is similar to topoisomerase V (Table 3). Lastly, a small protein with a distinct transmembrane domain, C92 (9.8 kDa, pI 5.19) was found in two spots that migrated only slightly slower than the dye front in the molecular weight dimension (Figure 1). In comparison to these proteomics results, the microarrays showed that all STIV ORFs were actively transcribed (Figure S1). Likely reasons for this discrepancy are low protein abundances for some of the viral transcripts, regulatory functions for RNAs that did not lead to significant translation, and possible incompatibility of some proteins with 2D gel analysis.

An exciting development in archaeal virology was the recent description of novel VAPs that appear on the surface of *Sulfolobus* prior to viral egress. Two recent publications, one looking at STIV in *Sulfolobus solfataricus* and another at the rod-shaped virus SIRV-2 that infects *Sulfolobus islandicus*, show that VAPs arise from the *Sulfolobus* cell surface and apparently lead to rupture of the tough protein-rich S-layer prior to cell lysis.^{8,9} The VAPs from SIRV were reported to be composed strictly of protein from SIRV ORF98.⁷⁸ The mechanism of formation for the VAPs is not known, but the dramatic rearrangements that take place in the S-layer and membrane suggest that proteins possessing membrane interaction domains will be important. A number of the highly expressed STIV proteins (e.g., A223, B130, B264, B345, C381 and C92) are predicted to contain transmembrane sections. Because membrane proteins are typically at low levels relative to cytoplasmic proteins and membrane proteins may behave poorly on standard 2D gels, a second approach was needed to look for changes in membrane protein abundance.

Membrane associated proteins

To enhance detection of membrane associated proteins and changes in protein composition, 1D SDS-PAGE analysis of enriched membrane fractions was carried out. A comparison of membrane protein fractions from mock and STIV infected cells showed that the overall 1D gel banding pattern was quite similar (Figure 3). However, there were several visually distinct differences, indicated by asterisks, such as the major band just below the 10-kD marker in the infected cell samples. Each lane was cut into 22 horizontal slices and subjected to in-gel proteolysis and protein identification. From the 22 pairs of samples, 74 host proteins were identified; 57 were common to both, 13 unique to infected, and 5 unique to control (for the complete list see Supplemental Table 1). Protein sequence analysis indicated that approximately 50% of these proteins were predicted to have transmembrane domains. Nine viral proteins were identified from the membrane fraction; A55, A61, A223, A510, B130, B164, B345, C92, and C381. Four of which (A55, A61, A510, and B164) were not found on the 2D gels.

C92 is of particular interest because it is believed to be responsible for VAP formation implicated in cell lysis and viral release.⁷⁹ It was highly expressed in the membrane fraction (Figure 3). Sequence analysis indicated that C92 has a single transmembrane helix and a soluble domain that is likely to be helical as well (Figure 4). The only proteins in the NCBI nonredundant library with sequence similarity are other crenarchaeal viral proteins; P98 from *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2); gp49 from *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2_gp49) and gp42 from *Sulfolobus islandicus* rod-shaped virus 1 (SIRV1_gp42) (Figure 4B). Recently it has been shown that SIRV2 virus infection of *S. islandicus* also produces VAPs akin to those from *S. solfataricus*²⁸ and that viral protein P98 is responsible for formation of the VAPs in the native archeal host as well as on *E. coli* bacteria after heterologous expression.^{78, 80}

The large quantity of C92 present in membrane fractions after STIV infection, suggested that the protein was present in quantities sufficient for VAP assembly. To investigate properties of the protein, C92 was expressed in *E. coli* as a full length or as a truncated construct. Full length C92 expressed to moderate levels and, as expected, partitioned with the membrane fraction of the cell lysate. Removal of the transmembrane spanning region, C92Δ1-27, produced a highly soluble protein and both the N and C terminal His-tagged constructs were readily purified.

It seems likely that the VAPs are partially or entirely formed by protein polymerization.^{78, 79} To test the oligomeric state of C92 and its potential to initiate polymerization, the purified recombinant protein lacking the TM domain (1–27) was analyzed by size exclusion chromatography (SEC). Based on the elution volume, the truncated C92 was present as a distribution of oligomeric species (Figure S5A). The lack of a strong propensity for the soluble portion of C92 to aggregate into megadalton size complexes suggests that either oligomerization is driven by the N-terminal membrane spanning region (residues 1–27) which was deleted, or that C92 acts in concert with other proteins and/or lipids in the cell.

To search for interaction partners of C92, the C-terminal domain was chemically linked to Sepharose resin and incubated with cell lysates from mock and infected *S. solfataricus* cells. After trying a number of binding and wash conditions, no proteins with a high affinity for C92 were identified by 1D SDS-PAGE or LCMS of a tryptic digest of potentially C92-bound proteins (data not shown). As a final check for oligomerization activity, *E. coli* cells expressing full length C92 were visualized by negative stain electron microscopy. Many of the cells expressing C92 had a very strange morphology, with large “horn-like” protrusions, not found on any of the control cells, suggesting that the C92 protein has an inherent ability to alter membranes (Figure S5B). A similar experiment using SIRV2 P98 lead to the observation of VAP-like structures in *E. coli*⁷⁸. Given that VAP formation would require high levels of C92 expression we compared relative viral mRNA levels during infection of the ATCC stock strain SP2 and strain SP2-2-12 used for this study. Interestingly, ATCC stock cells showed a lower level of C92 transcription relative to other viral transcripts (Figure S1) and are largely resistant to infection (data not shown).

Further investigation into the other virally encoded proteins that were detected revealed some noteworthy findings. BLAST analysis of A61 showed that it has significant similarity to proteins from the Crenarchaeal stygiolobus rod-shaped virus and CopG/DNA-binding domain-containing protein from *Metallosphaera sedula* DSM 5348. A61 was also one of only two proteins in common between STIV, STIV2, and SIRV2²⁸. Sequence analysis and protein fold recognition performed for A510 using DUF²⁶ and PHYRE²⁷, suggested a role as a topoisomerase/recombinase. The band in which A223 and C381 were found had an apparent molecular mass of 200 kDa on 1D SDS-PAGE reducing gels (Figure 3, lane I, band 2); however, their predicted masses are 24 kDa and 42 kDa, respectively. These proteins

have recently been shown to be part of the turrets in STIV⁸¹, confirming our earlier predictions based on a proteomic analysis of purified STIV¹⁴. The anomalously high molecular masses of A223 and C381 in 1D gel analysis suggest that they may reside in a complex that resists disruption in reducing SDS. This behavior has been described for similar structural proteins from mesophilic viruses, such as PBCV-1⁸². Under the conditions used for 2D gel analysis, the proteins were detected close to their expected masses (Figure. 1). This is consistent with the behavior of these proteins from highly purified STIV¹⁴ and rules out the possibility of read through translation which can occur in *S. solfataricus* (Cobucci-Ponzano 2010).

STIV genome

Recoding of genetic information is well documented in archaea, including the use of nonstandard amino acids, frame shifts, read-through of amber codons, and alternative sites for the initiation of transcription^{83, 84}. To assure that virally expressed proteins were not overlooked, we formatted our protein sequence database such that STIV ORFs began immediately after stop codons and allowed for read-through products of the amber codon, which is a common site of recoding in archaea⁸⁴. This led to the identification of a new viral protein from 2D spot 20 (Figure 1). This ORF has an amber stop codon after the first 9 residues, so it was not listed in the original genome annotation¹². This ORF has now been annotated B129 (see Figure 5 and Figure S11). A blast search using the amino acid sequence from B129 against STIV2, produced a significant hit to STIV2 A105 which is itself similar to STIV C118 (Supplemental Figure S12). This raises the question of why STIV would have two such genes when only one appears to be present in STIV2. Further sequence analysis failed to produce significant findings and the function of these proteins remains to be discovered.

The combined data from the 1 and 2D gels led to the detection of 13 virally encoded proteins (Table 3). When the ORFs of the detected proteins are viewed on a circular map of the viral genome, they cluster together from 9 to 6, as on a clock (Figure 5). The sequential order of these proteins is indicative of a shared transcriptional regulatory mechanism, which is common for viruses in general and those that replicate in *S. solfataricus*.^{22, 85} The temporal variation of RNA expression observed in the microarray experiment on STIV was limited, all the viral proteins detected come from the region of the genome that first appeared after 16 hours, which was on the later side. Because most of the proteins from this region of the viral genome are part of the particle, they should be produced in large quantities, increasing the probability of detection in proteomics experiments. These results are consistent with predictions based on sequence analysis and structural data that the ORFs from 6–9 o'clock are regulatory proteins. This is also consistent with our previous data in that transcripts from this region were the first to be detected by microarray^{1, 15, 16}. This expression scheme then appears to explain the location of C92 and A197, within the late genes, even though they are not structural proteins. A197 was predicted to be a glycosyltransferase, based on the structural model from crystallography¹⁵ with the major capsid protein, B345, as a primary target for glycosylation^{14, 15}. Lastly, the level and timing of C92 protein production are consistent with it having a structural role in VAP formation, as discussed earlier.

Conclusions

This is the first study to investigate viral infection in archaeal cells using an untargeted proteomics-based approach despite widespread interest in archaeal organisms and more recently the viruses that infect them. As such, this work brings clarity and significant insights to STIV and archaeal host-virus interactions. A surprisingly consistent connection was found between *S. solfataricus* proteins that were altered during viral infection and

eukaryotic proteins known to be involved with viral infection and egress. For example, general and specific stress response proteins that mediate redox status were found to be involved. The regulation of ESCRT-like proteins by viral infection in both archaeal and eukaryotic systems implies a deep evolutionary root for viral trafficking processes. It was recently shown that genome packaging leads to redistribution of STIV particles within cells^{8, 86}. Our data point to the ESCRT-like CdvB homolog, SSO0881, as the likely candidate for delivery of double stranded viral DNA for packaging and a second Cdv operon, SSO0909-9011 for particle transport. The regulation of myosin-like proteins, such as SSO2632, could also be involved with transport or serve as a structural protein for cytoplasmic reorganization or formation of the electron dense spheres associated with VAPs⁸. The STIV C92 protein is clearly involved with VAP formation, though the details on what triggers polymerization and lysis are still unclear. Future studies now have specific targets for genetic manipulation, in both *Sulfolobus* and STIV, to elucidate the mechanism of cell division, the nature of the cellular cytoskeleton in the crenarchaea, and how the VAPs are built.

The evolutionary relationship between STIV and viruses with bacterial and eukaryotic hosts has been primarily built on morphological similarities^{8, 13}. The untargeted proteomics methods used here further strengthen the mechanistic connections across domains and suggests targets for future studies. For example, the functional analysis of C92 and the discovery of B129 add two important new pieces to the puzzles of archaeal viruses. The involvement of trafficking, oxidation, and RNA modification systems are akin to those used by eukaryotic viruses, indicating that the investigation of archaeal virus-host interactions will provide evolutionary insights to viruses and biology across all domains of life.

In the bigger picture, this study emphasizes the complimentary rather than competitive nature of different omics approaches^{23, 29, 87}. By integrating data from the transcriptome, proteome, and activity assays significant biological meaning is emerging. The challenge now is in how best to properly weight data from different sources and which types of data provide the most added value.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

BB is funded by MCB 0646499 and MCB 1022481 from the National Science Foundation, the Center for Bio-Inspired Nanomaterials (Office of Naval Research grant #N00014-06-01-1016), and the Thermal Biology Institute (NASA grant NNG04GR46G) and NSF DEB-0936178 (M.J.Y) and EF-080220 (M.J.Y) We would like to thank the Murdock Charitable Trust and NIH Cobre 5P20RR02437 for support of the Mass Spectrometry Facility at MSU.

References

1. Lawrence CM, Menon S, Eilers BJ, Bothner B, Khayat R, Douglas T, Young MJ. Structural and Functional Studies of Archaeal Viruses. *Journal of Biological Chemistry*. 2009; 284(19):12599–12603. [PubMed: 19158076]
2. Douglas T, Fulton J, Bothner B, Lawrence M, Johnson JE, Young M. Genetics, biochemistry and structure of the archaeal virus STIV. *Biochemical Society Transactions*. 2009; 37:114–117. [PubMed: 19143613]
3. Zillig W, Prangishvilli D, Schleper C, Elferink M, Holz I, Albers S, Janekovic D, Gotz D. Viruses, plasmids and other genetic elements of thermophilic and hyperthermophilic Archaea. *FEMS Microbiology Reviews*. 1996; 18(2–3):225–236. [PubMed: 8639330]

4. Prangishvili D, Mochizuki T, Yoshida T, Tanaka R, Forterre P, Sako Y. Diversity of viruses of the hyperthermophilic archaeal genus *Aeropyrum*, and isolation of the *Aeropyrum pernix* bacilliform virus 1, APBV1, the first representative of the family Clavaviridae. *Virology*. 2010; 402(2):347–354. [PubMed: 20430412]
5. Butcher SJ, Happonen LJ, Redder P, Peng X, Reigstad LJ, Prangishvili D. Familial Relationships in Hyperthermo- and Acidophilic Archaeal Viruses. *Journal of Virology*. 2010; 84(9):4747–4754. [PubMed: 20164227]
6. Wiedenheft B, Stedman K, Roberto F, Willits D, Gleske AK, Zoeller L, Snyder J, Douglas T, Young M. Comparative genomic analysis of hyperthermophilic archaeal Fuselloviridae viruses. *J Virol*. 2004; 78(4):1954–61. [PubMed: 14747560]
7. Kessler A, Brinkman AB, van der Oost J, Prangishvili D. Transcription of the rod-shaped viruses SIRV1 and SIRV2 of the hyperthermophilic archaeon *Sulfolobus*. *J Bacteriol*. 2004; 186(22):7745–53. [PubMed: 15516589]
8. Brumfield SK, Ortmann AC, Ruigrok V, Suci P, Douglas T, Young MJ. Particle Assembly and Ultrastructural Features Associated with Replication of the Lytic Archaeal Virus *Sulfolobus* Turreted Icosahedral Virus. *Journal of Virology*. 2009; 83(12):5964–5970. [PubMed: 19357174]
9. Bize A, Karlsson EA, Ekefjard K, Quax TEF, Pina M, Prevost MC, Forterre P, Tenaillon O, Bernander R, Prangishvili D. A unique virus release mechanism in the Archaea. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(27):11306–11311. [PubMed: 19549825]
10. Prangishvili D, Vestergaard G, Haring M, Aramayo R, Basta T, Rachel R, Garrett RA. Structural and genomic properties of the hyperthermophilic archaeal virus ATV with an extracellular stage of the reproductive cycle. *Journal of Molecular Biology*. 2006; 359(5):1203–1216. [PubMed: 16677670]
11. Rice G, Stedman K, Snyder J, Wiedenheft B, Willits D, Brumfield S, McDermott T, Young MJ. Viruses from extreme thermal environments. *Proc Natl Acad Sci U S A*. 2001; 98(23):13341–5. [PubMed: 11606757]
12. Rice G, Tang L, Stedman K, Roberto F, Spuhler J, Gillitzer E, Johnson JE, Douglas T, Young M. The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(20):7716–7720. [PubMed: 15123802]
13. Khayat R, Tang L, Larson ET, Lawrence CM, Young M, Johnson JE. Structure of an archaeal virus capsid protein reveals a common ancestry to eukaryotic and bacterial viruses. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(52):18944–18949. [PubMed: 16357204]
14. Maaty WSA, Ortmann AC, Dlakic M, Schulstad K, Hilmer JK, Liepold L, Weidenheft B, Khayat R, Douglas T, Young MJ, Bothner B. Characterization of the archaeal thermophile *Sulfolobus* turreted icosahedral virus validates an evolutionary link among double-stranded DNA viruses from all domains of life. *Journal of Virology*. 2006; 80(15):7625–7635. [PubMed: 16840341]
15. Larson ET, Reiter D, Young M, Lawrence CM. Structure of A197 from *Sulfolobus* turreted icosahedral virus: a crenarchaeal viral glycosyltransferase exhibiting the GT-A fold. *Journal of Virology*. 2006; 80(15):7636–7644. [PubMed: 16840342]
16. Larson ET, Eilers B, Menon S, Reiter D, Ortmann A, Young MJ, Lawrence CM. A winged-helix protein from *Sulfolobus* turreted icosahedral virus points toward stabilizing disulfide bonds in the intracellular proteins of a hyperthermophilic virus. *Virology*. 2007; 368(2):249–261. [PubMed: 17669459]
17. Larson ET, Eilers BJ, Reiter D, Ortmann AC, Young MJ, Lawrence CM. A new DNA binding protein highly conserved in diverse crenarchaeal viruses. *Virology*. 2007; 363(2):387–396. [PubMed: 17336360]
18. Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, Prieur D. PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote, “*Pyrococcus abyssi*”. *Journal of Bacteriology*. 2003; 185(13):3888–3894. [PubMed: 12813083]
19. Martin A, Yeats S, Janekovic D, Reiter WD, Aicher W, Zillig W. Sav-1, a Temperate Uv-Inducible DNA Virus-Like Particle from the Archaeobacterium *Sulfolobus*-*Acidocaldarius* Isolate B-12. *Embo Journal*. 1984; 3(9):2165–2168. [PubMed: 16453555]

20. Prangishvili D, Garrett RA. Viruses of hyperthermophilic Crenarchaea. *Trends in Microbiology*. 2005; 13(11):535–542. [PubMed: 16154357]
21. Xiang XY, Chen LM, Huang XX, Luo YM, She QX, Huang L. *Sulfolobus tengchongensis* spindle-shaped virus STSV1: Virus-host interactions and genomic features. *Journal of Virology*. 2005; 79(14):8677–8686. [PubMed: 15994761]
22. Ortmann AC, Brumfield SK, Walther J, McInnerney K, Brouns SJJ, van de Werken HJG, Bothner B, Douglas T, van de Oost J, Young MJ. Transcriptome analysis of infection of the Archaeon *Sulfolobus solfataricus* with *Sulfolobus* turreted icosahedral virus. *Journal of Virology*. 2008; 82(10):4874–4883. [PubMed: 18337566]
23. Feder ME, Walser JC. The biological limitations of transcriptomics in elucidating stress and stress responses. *Journal of Evolutionary Biology*. 2005; 18(4):901–910. [PubMed: 16033562]
24. Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Shevchenko A, Boucherie H, Mann M. Linking genome and proteome by mass spectrometry: Large-scale identification of yeast proteins from two dimensional gels. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93(25):14440–14445. [PubMed: 8962070]
25. Lower BH, Bischoff KM, Kennelly PJ. The archaeon *Sulfolobus solfataricus* contains a membrane-associated protein kinase activity that preferentially phosphorylates threonine residues in vitro. *Journal of Bacteriology*. 2000; 182(12):3452–3459. [PubMed: 10852877]
26. Dlakic M. HHsvm: fast and accurate classification of profile-profile matches identified by HHsearch. *Bioinformatics*. 2009; 25(23):3071–3076. [PubMed: 19773335]
27. Kelley LA, Sternberg MJE. Protein structure prediction on the Web: a case study using the Phyre server. *Nature Protocols*. 2009; 4(3):363–371.
28. Happonen LJ, Redder P, Peng X, Reigstad LJ, Prangishvili D, Butcher SJ. Familial Relationships in Hyperthermo- and Acidophilic Archaeal Viruses. *Journal of Virology*. 2010; 84(9):4747–4754. [PubMed: 20164227]
29. Maaty WS, Wiedenheft B, Tarlykov P, Schaff N, Heinemann J, Robison-Cox J, Valenzuela J, Dougherty A, Blum P, Lawrence CM, Douglas T, Young MJ, Bothner B. Something Old, Something New, Something Borrowed: How the Thermoacidophilic Archaeon *Sulfolobus solfataricus* Responds to Oxidative Stress. *Plos One*. 2009; 4(9)
30. Majek P, Reicheltova Z, Stikarova J, Suttner J, Sobotkova A, Dyr JE. Proteome changes in platelets activated by arachidonic acid, collagen, and thrombin. *Proteome Science*. 2010; 8
31. Barry RC, Young MJ, Stedman KM, Dratz EA. Proteomic mapping of the hyperthermophilic and acidophilic archaeon *Sulfolobus solfataricus* P2. *Electrophoresis*. 2006; 27(14):2970–2983. [PubMed: 16721906]
32. Attia N, Rachez C, De Pauw A, Avner P, Rogner UC. Nap112 promotes histone acetylation activity during neuronal differentiation. *Molecular and Cellular Biology*. 2007; 27(17):6093–6102. [PubMed: 17591696]
33. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128(4):693–705. [PubMed: 17320507]
34. Mancebo HSY, Lee G, Flygare J, Tomassini J, Luu P, Zhu YR, Peno JM, Blau C, Hazuda D, Price D, Flores O. P-TEFb kinase is required for HIV Tat transcriptional activation in vivo and in vitro. *Genes & Development*. 1997; 11(20):2633–2644. [PubMed: 9334326]
35. Benkirane M, Chun RF, Xiao H, Ogryzko VV, Howard BH, Nakatani Y, Jeang KT. Activation of integrated provirus requires histone acetyltransferase - p300 AND P/CAF are coactivators for HIV-1 Tat. *Journal of Biological Chemistry*. 1998; 273(38):24898–24905. [PubMed: 9733796]
36. Col E, Caron C, Seigneurin-Berny D, Gracia J, Favier A, Khochbin S. The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator, Tat. *Journal of Biological Chemistry*. 2001; 276(30):28179–28184. [PubMed: 11384967]
37. Marzio G, Tyagi M, Gutierrez MI, Giacca M. HIV-1 Tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95(23):13519–13524. [PubMed: 9811832]

38. Rehtanz M, Schmidt HM, Warthorst U, Steger G. Direct interaction between nucleosome assembly protein 1 and the papillomavirus E2 proteins involved in activation of transcription. *Molecular and Cellular Biology*. 2004; 24(5):2153–2168. [PubMed: 14966293]
39. Wang QJ, Zhang YK, Yang C, Xiong H, Lin Y, Yao J, Li H, Xie L, Zhao W, Yao YF, Ning ZB, Zeng R, Xiong Y, Guan KL, Zhao SM, Zhao GP. Acetylation of Metabolic Enzymes Coordinates Carbon Source Utilization and Metabolic Flux. *Science*. 2010; 327(5968):1004–1007. [PubMed: 20167787]
40. Zhao SM, Xu W, Jiang WQ, Yu W, Lin Y, Zhang TF, Yao J, Zhou L, Zeng YX, Li H, Li YX, Shi J, An WL, Hancock SM, He FC, Qin LX, Chin J, Yang PY, Chen X, Lei QY, Xiong Y, Guan KL. Regulation of Cellular Metabolism by Protein Lysine Acetylation. *Science*. 2010; 327(5968): 1000–1004. [PubMed: 20167786]
41. Samson RY, Obita T, Freund SM, Williams RL, Bell SD. A Role for the ESCRT System in Cell Division in Archaea. *Science*. 2008; 322(5908):1710–1713. [PubMed: 19008417]
42. Lindas AC, Karlsson EA, Lindgren MT, Ettema TJG, Bernander R. A unique cell division machinery in the Archaea. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(48):18942–18946. [PubMed: 18987308]
43. Bernander R, Lundgren M, Ettema TJG. Comparative and functional analysis of the archaeal cell cycle. *Cell Cycle*. 2010; 9(4):795–806.
44. McDonald B, Martin-Serrano J. No strings attached: the ESCRT machinery in viral budding and cytokinesis. *Journal of Cell Science*. 2009; 122(13):2167–2177. [PubMed: 19535732]
45. Corless L, Crump CM, Griffin SDC, Harris M. Vps4 and the ESCRT-III complex are required for the release of infectious hepatitis C virus particles. *Journal of General Virology*. 2010; 91:362–372. [PubMed: 19828764]
46. Ellen AF, Albers SV, Huibers W, Pitcher A, Hobel CFV, Schwarz H, Folea M, Schouten S, Boekema EJ, Poolman B, Driessen AJM. Proteomic analysis of secreted membrane vesicles of archaeal *Sulfolobus* species reveals the presence of endosome sorting complex components. *Extremophiles*. 2009; 13(1):67–79. [PubMed: 18972064]
47. Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Cote M, Rich RL, Myszkowski DG, Sundquist WL. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*. 2001; 107(1):55–65. [PubMed: 11595185]
48. Martin-Serrano J, Zang T, Bieniasz PD. HIV-I and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nature Medicine*. 2001; 7(12): 1313–1319.
49. Taylor GM, Hanson PI, Kielian M. Ubiquitin depletion and dominant-negative VPS4 inhibit rhabdovirus budding without affecting alphavirus budding. *Journal of Virology*. 2007; 81(24): 13631–13639. [PubMed: 17913808]
50. Crump CA, Yates C, Minson T. Herpes simplex virus type 1 cytoplasmic envelopment requires functional Vps4. *Journal of Virology*. 2007; 81(14):7380–7387. [PubMed: 17507493]
51. Snyder JC, Young MJ. Potential role of cellular ESCRT proteins in the STIV life cycle. *Biochemical Society Transactions*. 2011; 39:107–110. [PubMed: 21265756]
52. Jansen RP, Hurt EC, Kern H, Lehtonen H, Carmofonseca M, Lapeyre B, Tollervey D. Evolutionary Conservation of the Human Nucleolar Protein Fibrillarin and Its Functional Expression in Yeast. *Journal of Cell Biology*. 1991; 113(4):715–729. [PubMed: 2026646]
53. Ponti D, Troiano M, Bellenchi GC, Battaglia PA, Gigliani F. The HIV Tat protein affects processing of ribosomal RNA precursor. *Bmc Cell Biology*. 2008; 9
54. Yoo D, Wootton SK, Li G, Song C, Rowland RR. Colocalization and interaction of the porcine arterivirus nucleocapsid protein with the small nucleolar RNA-associated protein fibrillarin. *Journal of Virology*. 2003; 77(22):12173–12183. [PubMed: 14581554]
55. Yuan XL, Yao ZY, Shan YJ, Chen B, Yang Z, Wu J, Zhao ZH, Chen JP, Cong YW. Nucleolar localization of non-structural protein 3b, a protein specifically encoded by the severe acute respiratory syndrome coronavirus. *Virus Research*. 2005; 114(1–2):70–79. [PubMed: 16046244]
56. Lymberopoulos MH, Pearson A. Involvement of UL24 in herpes-simplex-virus-1-induced dispersal of nucleolin. *Virology*. 2007; 363(2):397–409. [PubMed: 17346762]

57. Kim SH, MacFarlane S, Kalinina NO, Rakitina DV, Ryabov EV, Gillespie T, Haupt S, Brown JWS, Taliansky M. Interaction of a plant virus-encoded protein with the major nucleolar protein fibrillarin is required for systemic virus infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(26):11115–11120. [PubMed: 17576925]
58. Kim SH, Ryabov EV, Kalinina NO, Rakitina DV, Gillespie T, MacFarlane S, Haupt S, Brown JWS, Taliansky M. Cajal bodies and the nucleolus are required for a plant virus systemic infection. *Embo Journal*. 2007; 26(8):2169–2179. [PubMed: 17410203]
59. Pedone E, Limauro D, D'Ambrosio K, De Simone G, Bartolucci S. Multiple catalytically active thioredoxin folds: a winning strategy for many functions. *Cellular and Molecular Life Sciences*. 2010; 67(22):3797–3814. [PubMed: 20625793]
60. Limauro D, Pedone E, Galdi I, Bartolucci S. Peroxiredoxins as cellular guardians in *Sulfolobus solfataricus* - characterization of Bcp1, Bcp3 and Bcp4. *Febs Journal*. 2008; 275(9):2067–2077. [PubMed: 18355320]
61. Pedone E, Limauro D, D'Alterio R, Rossi M, Bartolucci S. Characterization of a multifunctional protein disulfide oxidoreductase from *Sulfolobus solfataricus*. *Febs Journal*. 2006; 273(23):5407–5420. [PubMed: 17076700]
62. Kalantari P, Narayan V, Natarajan SK, Muralidhar K, Gandhi UH, Vunta H, Henderson AJ, Prabhu KS. Thioredoxin Reductase-1 Negatively Regulates HIV-1 Transactivating Protein Tat-dependent Transcription in Human Macrophages. *Journal of Biological Chemistry*. 2008; 283(48):33183–33190. [PubMed: 18835810]
63. Rhee SG, Chae HZ, Kim K. Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radical Biology and Medicine*. 2005; 38(12):1543–1552. [PubMed: 15917183]
64. Limauro D, Pedone E, Pirone L, Bartolucci S. Identification and characterization of 1-Cys peroxiredoxin from *Sulfolobus solfataricus* and its involvement in the response to oxidative stress. *Febs Journal*. 2006; 273(4):721–731. [PubMed: 16441659]
65. She Q, Singh RK, Confalonieri F, Zivanovic Y, Allard G, Awayez MJ, Chan-Weiher CCY, Clausen IG, Curtis BA, De Moors A, Erauso G, Fletcher C, Gordon PMK, Heikamp-de Jong I, Jeffries AC, Kozera CJ, Medina N, Peng X, Thi-Ngoc HP, Redder P, Schenk ME, Theriault C, Tolstrup N, Charlebois RL, Doolittle WF, Duguet M, Gaasterland T, Garrett RA, Ragan MA, Sensen CW, Van der Oost J. The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98(14):7835–7840. [PubMed: 11427726]
66. Li S, Qu H, Hao JW, Sun JF, Guo HC, Guo CM, Sun BX, Tu CC. Proteomic analysis of primary porcine endothelial cells after infection by classical swine fever virus. *Biochimica Et Biophysica Acta-Proteins and Proteomics*. 2010; 1804(9):1882–1888.
67. Jamaluddin M, Wiktorowicz JE, Soman KV, Boldogh I, Forbus JD, Spratt H, Garofalo RP, Brasier AR. Role of Peroxiredoxin 1 and Peroxiredoxin 4 in Protection of Respiratory Syncytial Virus-Induced Cysteinyl Oxidation of Nuclear Cytoskeletal Proteins. *Journal of Virology*. 2010; 84(18):9533–9545. [PubMed: 20610706]
68. Oh SJ, Chae J, Zhu H, Hien TT, Lee K, Kim HM, Kang KW, Song GY, Kang JS, Kim BH, Kwon KI, Kim SK. Evaluation of antioxidant defense systems in H4IIE cells infected with a retroviral vector. *Toxicology in Vitro*. 2010; 24(4):1105–1110. [PubMed: 20332021]
69. Shaw J, Rowlinson R, Nickson J, Stone T, Sweet A, Williams K, Tonge R. Evaluation of saturation labelling two-dimensional difference gel electrophoresis fluorescent dyes. *Proteomics*. 2003; 3(7):1181–1195. [PubMed: 12872219]
70. Chaban B, Voisin S, Kelly J, Logan SM, Jarrell KF. Identification of genes involved in the biosynthesis and attachment of *Methanococcus voltae* N-linked glycans: insight into N-linked glycosylation pathways in Archaea. *Molecular Microbiology*. 2006; 61(1):259–268. [PubMed: 16824110]
71. Krishna RG, Wold F. Posttranslational Modification of Proteins. *Advances in Enzymology and Related Areas of Molecular Biology*. 1993; 67:265–298. [PubMed: 8322616]
72. Chambers JW, Maguire TG, Alwine JC. Glutamine Metabolism Is Essential for Human Cytomegalovirus Infection. *Journal of Virology*. 2010; 84(4):1867–1873. [PubMed: 19939921]

73. Munger J, Bajad SU, Collier HA, Shenk T, Rabinowitz JD. Dynamics of the cellular metabolome during human cytomegalovirus infection. *Plos Pathogens*. 2006; 2(12):1165–1175.
74. Munger J, Bennett BD, Parikh A, Feng XJ, McArdle J, Rabitz HA, Shenk T, Rabinowitz JD. Systems-level metabolic flux profiling identifies fatty acid synthesis as a target for antiviral therapy. *Nature Biotechnology*. 2008; 26(10):1179–1186.
75. Duin EC, Madadi-Kahkesh S, Hedderich R, Clay MD, Johnson MK. Heterodisulfide reductase from *Methanothermobacter marburgensis* contains an active-site [4Fe-4S] cluster that is directly involved in mediating heterodisulfide reduction. *Febs Letters*. 2002; 512(1–3):263–268. [PubMed: 11852093]
76. Shokes JE, Duin EC, Bauer C, Jaun B, Hedderich R, Koch J, Scott RA. Direct interaction of coenzyme M with the active-site Fe-S cluster of heterodisulfide reductase. *Febs Letters*. 2005; 579(7):1741–1744. [PubMed: 15757669]
77. Weller SK, Albright BS, Nellissery J, Szczepaniak R. Disulfide Bond Formation in the Herpes Simplex Virus 1 UL6 Protein Is Required for Portal Ring Formation and Genome Encapsidation. *Journal of Virology*. 2011; 85(17):8616–8624. [PubMed: 21593161]
78. Quax TEF, Lucas S, Reimann J, Pehau-Arnaudet G, Prevost MC, Forterre P, Albers SV, Prangishvili D. Simple and elegant design of a virion egress structure in Archaea. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(8):3354–3359. [PubMed: 21282609]
79. Snyder JC, Brumfield SK, Peng N, She Q, Young MJ. Sulfolobus Turreted Icosahedral Virus c92 protein responsible for formation of pyramid-like cellular lysis structures. *Journal of Virology*. 2011; 85(13):6287–6292. [PubMed: 21525343]
80. Prangishvili D, Quax TEF. Exceptional virion release mechanism: one more surprise from archaeal viruses. *Current Opinion in Microbiology*. 2011; 14(3):315–320.
81. Khayat R, Fu CY, Ortmann AC, Young MJ, Johnson JE. The Architecture and Chemical Stability of the Archaeal Sulfolobus Turreted Icosahedral Virus. *Journal of Virology*. 2010; 84(18):9575–9583. [PubMed: 20592081]
82. Nandhagopal N, Simpson AA, Gurnon JR, Yan XD, Baker TS, Graves MV, Van Etten JL, Rossmann MG. The structure and evolution of the major capsid protein of a large, lipid-containing DNA virus. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99(23):14758–14763. [PubMed: 12411581]
83. Cobucci-Ponzano B, Rossi M, Moracci M. Recoding in Archaea. *Molecular Microbiology*. 2005; 55(2):339–348. [PubMed: 15659155]
84. Ibba M, Soll D. Genetic code: Introducing pyrrolysine. *Current Biology*. 2002; 12(13):R464–R466. [PubMed: 12121639]
85. Palm P, Schleper C, Grampp B, Yeats S, McWilliam P, Reiter WD, Zillig W. Complete Nucleotide-Sequence of the Virus Ssv1 of the Archaeobacterium Sulfolobus-Shibatae. *Virology*. 1991; 185(1):242–250. [PubMed: 1926776]
86. Fu CY, Wang K, Gan L, Lanman J, Khayat R, Young MJ, Jensen GJ, Doerschuk PC, Johnson JE. In Vivo Assembly of an Archaeal Virus Studied with Whole-Cell Electron Cryotomography. *Structure*. 2010; 18(12):1579–1586. [PubMed: 21134637]
87. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Molecular and Cellular Biology*. 1999; 19(3):1720–1730. [PubMed: 10022859]

Synopsis

On the surface, the archaeal hyperthermo acidiphile *Sulfolobus solfataricus* and the virus STIV, appear to be very different from eukaryotic virus-host pairs. After conducting a thorough proteomics analysis, we conclude that the difference is only skin-deep. Below the unique viral associated pyramids, the activated replication, transport, and redox systems are similarly involved in eukaryotic viral infection.

Figure 1.

2D gel of the *S. solfataricus* strain 2-2-12 proteome 32 hpi with STIV. Approximately 800 protein spots were common in all samples with CyDye-based labeling and described in the text with analysis of three biological replicates. Twenty protein spots changed significantly in abundance. These spots are indicated on the gel image and protein identifications were made using in-gel proteolysis followed by LC-MS/MS (Table 1 & 2). * Asterisk indicates spots that contain STIV protein.

Figure 2. Host protein thiol reactivity changes during STIV infection. A maleimide probe linked to a novel zwitterionic dye, ZB-maleimide (ZBBM) (C), was reacted with total soluble protein from *S. solfataricus* at 24 (A) and 32 (B) hpi. Approximately 300 protein spots were labeled with the cysteine specific probe. Subsequent staining for total protein, using SyproRuby, showed ~800 spots (as in Figure 1). DIGE analysis of the 2D gels found 10 protein spots that had a statistically significant change in thiol labeling. Spots that were altered indicated by numbers. Protein identifications were made using in-gel proteolysis followed by LC-MS/MS (see Table 3). (* altered spots were the protein concentration was below in-gel digest LCMS detection limit). **D.** Cysteine distribution in the *S. solfataricus* proteome based on Uniport database annotation.

Figure 3.

Membrane proteins from infected and mock infected *Sulfolobus* SP2 cells. **A.** 50 μ g of membrane final pellet fraction from infected (I) and mock-infected (M) cells were resolved over 4–20% SDS-PAGE. Numbered bands were cut from each corresponding lane and in-gel tryptic digestions were performed. **B.** Band intensity profile for the lanes in panel (A) showing overlaid intensity of infected (black line) and mock-infected cells (gray line). Peaks and bands labeled with asterisks indicate major differences and STIV proteins. Complete list of identified proteins is in supplemental Table 1.

Figure 4. STIV C92 Transmembrane prediction diagram, secondary structure prediction and sequence alignment for its homologues from other archaeal viruses. **A.** The membrane TMHMM posterior probabilities for C92 and its homologue SIRV2-gp49. Transmembrane prediction of the STIV genome revealed the presence of a single transmembrane domain (shown by bar). C92 may be involved in pyramid-like structure formation and viral release. A protein of similar size and biochemical composition from SIRV2 was found to form a similar Pyramidal-like structure in an archaeal infection study ⁹. **B.** Multiple sequence alignment for SIRV2-P98, SIRV1-gp42, SIRV2 gp49 and STIV-C92 showing a high similarity to each other. **C.** Secondary structure prediction using CLUSTAL 2.1 shows largely alpha helix configuration among the 4 proteins.

Figure 5.

The STIV genome map (AY569307). Open reading frames (ORFs) for the 12 proteins that were detected during the viral infection cycle are shown with their reading frame codes and numbers. The nine ORFs that encode the particle associated proteins are labeled with asterisks. STIV ORFs are named according to reading frame (A to F) and number of predicted amino acids. The 12 detected virus proteins cluster on the genome map between 9 and 6 o'clock, which may indicate an operon-like organization. Seven of the nine proteins identified from purified virus particles¹⁴ were identified by their increased abundance in the proteomics experiments, the two capsid associated proteins (A78 and C557) not found in this study are indicated by asterisked unshaded ORFs. Virus proteins found in the membrane (gray), cytoplasm (light gray) or both fractions are indicated in black.

Table 1

Sulfolobus solfataricus proteins regulated by STIV infection

Protein Name	Locus Name	Spot	Mass (Da)	pI	Fold Difference	Unique Peptides	Cellular Role Category
Acetyltransferase, putative	SSO0209	16	25263	8.3	10.7	2	Acetyltransferases, Central intermediary metabolism
ESCRT-like ^a	SSO0881	10	25020	5.9	8.0	4	Conserved protein implicated in secretion (VPS24) (CdvB homologs of eukaryotic ESCRT-III) & DNA replication
NAD-dependent malic enzyme (malate oxidoreductase)	SSO2869	1	47967	6.0	8.0	5	Energy production and conversion: TCA cycle and other
ESCRT-like ^{a,b}	SSO0451	11	31785	6.1	8.0	1	Conserved protein implicated in secretion (VPS24)(cdvB homolog of eukaryotic ESCRT-III) & DNA replication
Hypothetical protein (myosin/tropomyosin like)	SSO2632	8	42203	4.9	5.3	2	Cytoskeletal cell division protein
Conserved hypothetical protein (myosin/tropomyosin)	SSO0911	11	30522	5.9	4.0	2	CdvA homolog of eukaryotic ESCRT-III & DNA replication
Fibrillarin (pre-rRNA processing protein)	SSO0940	12	26520	7.7	3.7	5	Fibrillarin-like rRNA methylase Transcription: RNA processing
Adenylate kinase (adkA)	SSO0694	14	21313	7.9	2.1	5	Nucleotide transport and metabolism
Disulfide Oxidoreductase	SSO0192	13	26358	4.7	1.8	8	Posttranslational modification, protein turnover, chaperones
Peroxisome, bacterioferritin comigratory protein homolog (bcp-4)	SSO2613	15	17564	7.7	-2.9	2	Posttranslational modification, protein turnover, chaperones

^aThis gene is listed as a conserved hypothetical in TIGR and NCBI databases.

^bProtein identification based on sequence data from a single peptide that was observed repeatedly. For details, see supplemental material Figure S10.

Table 2

Host proteins displaying differential cysteine reactivity

SSO	Spot #	Protein	GI	Cysteine No.	Fold difference	Mass	Score	PI	Peptides
SSO2044	8	Glutamate dehydrogenase [1.4.1.3]	gil13815328	1	4.8	46034	626	6.5	5
SSO0421	13	Transitional endoplasmic reticulum ATPase (CDC48-1)	gil13813572	5	4.6	86293	260	5.77	4
SSO0421	18	Transitional endoplasmic reticulum ATPase (CDC48-1)	gil13813572	5	4.8	86293	430	5.77	4
SSO0564	29	ATP synthase subunit B (atpB)	gil13813730	1	-3.3	51057	94	5.3	6
SSO0282	29	Thermosome beta subunit (thermophilic factor 55)	gil13813422	1	-3.3	60330	92.41	5.56	3
SSO0564	35	ATP synthase subunit B (atpB)	gil13813730	1	-3.1	51057	171	5.3	5
SSO1151	35	Conserved hypothetical protein, tldD protein, putative	gil13814340	1	-3.1	51678	145	5.17	3
SSO1134	60	Heterodisulfide reductase subunit C (hdrC-2)	gil13814322	8	-3.6	25654	96.3	6.24	2

Table 3

Sulfolobus turreted icosahedral virus (STIV) expressed proteins

Protein Name	Mass (Da)	pI	Transmembrane ^a	1 D band	2D Spot	Predicted structure and cellular function	Cellular location	1D # of peptides	2D # of peptides
A223	24,410	4.72	2	2	13	PRD1 P5 vertex protein	Cytoplasm & membrane & Vp ^b	3	4
A510	60,088	9.27	0	22	ND ^c	Topoisomerase/recombinase	Membrane	6	0
A55 ^d	6,211	4.49	1	21	ND ^c	NSM, similar to STIV hypothetical protein C67	Membrane & Vp ^b	1	0
A61 ^d	7,375	8.01	0	22	ND ^c	Similar to hypothetical protein SSO10342; CopG/DNA-binding domain-containing protein <i>Metallosphaera s.</i> ; hypothetical protein [Stygiolobus rod-shaped virus]	Membrane	1	0
A78	9,610	10.79	0	ND ^c	ND ^c	NSM ^e	Vp ^{b,f}	0	0
B109	11,969	5.04	0	ND ^c	2, 3	NSM ^e	Cytoplasm & Vp ^b	0	4
B116	13,437	4.79	0	ND ^c	17	Domain of unknown function (DUF1874), similar to hypothetical protein SIRV2gp37 & SIRV1gp29	Cytoplasm	0	2
B129 ^g	13,232	5.08	0	ND ^c	20	Similar to STIV2 hypothetical protein A105	Cytoplasm	0	2
B130 ^d	13,768	4.97	2	21, 22	19, 20	NSM ^e	Membrane & Vp ^b	1	1
B164 ^d	19,025	9.24	0	17	ND ^c	Similar to putative nucleotidyltransferase <i>Methanosarcina m.</i>	Membrane & Vp ^b	1	0
B264	30,214	4.79	2	ND	9	Topoisomerase v	Cytoplasm	0	3
B345	37,810	6.17	3	12	4, 5, 6	Major Capsid Protein	Cytoplasm & Vp ^b	2	7
C381	41,712	5.56	4	2	7	PRD1 P5 vertex protein; similar to Bap-like [<i>Trichomonas vaginalis</i> G3]	Cytoplasm & membrane & Vp ^b	1	4
C557	58,574	5.50	2	ND ^c	ND ^c	Protein interaction	Vp ^{b,f}	0	0
C92	9,829	5.18	1	1, 7, 21, 22	18, 20	Similar to Sulfolobus islandicus rod-shaped virus 1 SIRV1gp42	Cytoplasm & membrane	4	5

^aTransmembrane prediction using Tmpred program, http://www.ch.embnet.org/software/TMPRED_form.html & TMHMM Server v. 2.0 <http://www.cbs.dtu.dk/services/TMHMM/>

^bVp = purified virus particles;

^cProteins only found in Vp1⁴.

^dNot detected;

Proteins identification based on sequence data from a single peptide that was observed repeatedly. For details, see supplemental material Figures S6-S9.

^cNo significant match;

^gNewly identified ORF