Potential role of cellular ESCRT proteins in the STIV life cycle

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Abstract
We are examining the archaeal virus STIV (Sulfolobus turreted icosahedral virus) in order to elucidate the details of its replication cycle and its interactions with its cellular host, Sulfolobus solfataricus. Infection of Sulfolobus by STIV initiates an unusual cell lysis pathway. One component of this pathway is the formation of pyramid-like structures on the surface of infected cells. Multiple seven-sided pyramid-like structures are formed on infected cells late in the STIV replication cycle. These pyramid-like structures are formed at sites where the Sulfolobus S-layer has been disrupted and through which the cellular membrane protrudes. It is through the pyramid-like structures that virus-induced cell lysis occurs in the final stages of the STIV replication cycle. The pathway and process by which these unusual lysis structures are produced appears to be novel to archaeal viruses and are not related to the well-characterized lysis mechanisms utilized by bacterial viruses. We are interested in elucidating both the viral and cellular components involved with STIV lysis of its infected cell. In particular, we are examining the potential role that Sulfolobus ESCRT (endosomal sorting complex required for transport)-like proteins play during viral infection and lysis. We hypothesize that STIV takes advantage of the Sulfolobus ESCRT machinery for virus assembly, transport and cellular lysis.

Introduction
Little is known about the viruses of Archaea when compared with the viruses infecting Eukarya and Bacteria. To date, 41 archaeal viruses have been described, in contrast with the more than 5000 bacterial and eukaryotic viruses that have been discovered. This lack of knowledge about archaeal viruses is not a reflection of their low prevalence in Nature, but rather a result of the few attempts that have been made to isolate archaeal viruses and the unique habitats in which many members of the Archaea and their viruses replicate. Traditionally, studies of virus-host organisms is performed in culture; however, the extreme habitats in which many members of the Archaea and their viruses replicate often makes culture-dependent studies difficult. Recently, with the application of viral metagenomic approaches, many novel archaeal virus genome sequences have been detected. However, without purified virus and the host organism maintained in culture, it is difficult to perform studies that will reveal more about virus-host interactions.

YNP (Yellowstone National Park) is an ideal environment to isolate and study archaeal viruses. For several years, we have studied the high-temperature (>75°C) acidic (pH < 4) environments within YNP where Archaea and their viruses dominate [1]. In these environments, archaeal hosts belonging to the crenarchaeal family Sulfolobales and their viruses are commonly found. We have examined several of the archaeal viruses that infect the members of Sulfolobales that we have isolated from YNP hot springs [1–5]. One such virus is STIV (Sulfolobus turreted icosahedral virus). STIV was originally isolated from a Sulfolobus host present in an acidic (pH 2.2) hot spring (82°C) within the Rabbit Creek thermal area of YNP [2]. STIV contains a circular double-stranded DNA genome of 17663 bp [2]. The virus encodes 37 ORFs (open reading frames), of which nine encode proteins identified in purified virus particles. Two cellular proteins have also been co-purified with virus particles [6]. Most of the STIV ORFs have no sequence similarity to other genes in the public databases. In order to gain insights into the possible function of STIV gene products, several STIV proteins have been examined by X-ray crystallography [2,7–10]. The STIV virion has also been examined by a combination of X-ray crystallography of the major capsid protein [10], cryo-electron microscopy and image reconstruction of the purified virus particles [2,11], and electron microscopy tomography of infected cells [12]. STIV is an icosahedral virus built upon a pseudo-T = 31 lattice with turret-like appendages at each of the 12 5-fold axes [2]. The architectural organization of STIV is similar to that of other viruses that infect hosts within the domains Bacteria (PRD1) and Eukarya [human adenovirus and the algal virus PBCV1 (Paramecium bursaria Chlorella virus 1)], suggesting a common ancient evolutionary origin for all of these viruses [2]. The turret-like structures on the 5-fold axes of the virus particle have been hypothesized to play a role in host recognition. STIV also possesses an internal membrane sandwiched between the viral genomic DNA and the major coat protein. The internal lipid layer found in the

Key words: endosomal sorting complex required for transport (ESCRT), Sulfolobus turreted icosahedral virus (STIV), Sulfolobus virus, virus-host interaction

Abbreviations used: AAA+, ATPase associated with various cellular activities; Alix, ALG-2 (apoptosis-linked gene 2)-interacting protein X; ESCRT, endosomal sorting complex required for transport; HCV, hepatitis C virus; hpi, hours post-infection; ORF, open reading frame; PBCV1, Paramecium bursaria Chlorella virus 1; PRD1, Sulfolobus islandicus rod-shaped virus 2; STIV, Sulfolobus turreted icosahedral virus; Vps4, vacuolar protein sorting 4; YNP, Yellowstone National Park.

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mature virus particle is enriched in a subpopulation of the lipids that are derived from the *Sulfolobus* cell membrane [6]. The major capsid protein contains a basic C-terminal helix that interacts with the inner lipid layer anchoring the exterior virion protein shell. The biological function of the internal lipid layer in STIV particles is unknown, but is likely to play a role in protecting the viral genome from acid hydrolysis. In addition, the internal membrane is also likely to play a role in viral assembly or disassembly.

**STIV–Sulfolobus interactions**

A suite of tools have been developed to probe STIV–Sulfolobus interactions. These include the development of a STIV genetic system, transcriptome analysis and proteomic analysis of STIV-infected cells. The ability to discover the function of unknown viral proteins has been greatly facilitated by the development of a STIV genetic system to create viral mutations and analysis of the resultant phenotypes. In order to accomplish this, a STIV infectious clone has been constructed [13]. We have created mutants for several of the STIV ORFs and are in the process of analysing their phenotypes. To understand further archaeal viral replication and host response to virus infection, transcriptomics studies were used to compare STIV-infected *Sulfolobus* with non-infected *Sulfolobus* cells. Using a custom microarray containing oligonucleotide probes to all ORFs found in both STIV and its host *Sulfolobus solfataricus* P2, the effect on viral and host transcription were determined in a near-synchronous single viral replication cycle [14]. The transcription of viral genes was first detected at 8 hpi (hours post-infection) with a peak in transcription at 24 hpi. The lysis of infected host cells was detected at 32 hpi, making STIV the first lytic crenarchaeal virus. Unlike most other DNA viruses, there was no obvious distinction between early and late viral gene transcription. Over the course of a viral infection, 124 host genes were up-regulated and 53 host genes were down-regulated. Many of the up-regulated genes were associated with DNA replication and repair, suggesting that, like many eukaryal viruses, STIV uses its host’s machinery to replicate the viral genome. The down-regulated genes were generally associated with energy production and metabolism. Interestingly, one of the most up-regulated gene sets was a hypothetical Vps4 (vacuolar protein sorting 4) AAA+ (ATPase associated with various cellular activities) and ESCRT (endosomal sorting complex required for transport)-III (S. solfataricus gene numbers SSO0910 and SSO0911) operon. This observation suggested a role for archaeal ESCRT-like proteins in completing the STIV replication cycle.

A proteomics approach of both purified STIV particles and STIV-infected cells was undertaken. Proteomic analysis of purified virus particles resulted in the identification of nine virally encoded proteins and two cellular proteins [6]. The proteins identified in the viral particles are co-localized on the STIV genetic map (Figure 1). The proteins that compose the virion turrets on the 5-fold axes have been predicted through this analysis [6]. We are currently working on mutating the protein components of the STIV turrets to see whether we can elucidate the function of individual proteins. As mentioned previously, two host proteins were found to consistently co-purify with STIV virus particles [6]. The first protein was SSO7D, which is a small basic DNA-binding protein. It is hypothesized that this protein may play a role in the packaging and condensing of the viral genome. The second host protein that co-purified with STIV particles was SSO881. This is a hypothetical protein that shows similarity to yeast and mammalian ESCRT-associated Vps24 proteins. Vps24 proteins are involved in cell sorting and trafficking. This result again points to archaeal ESCRT-like proteins being necessary for STIV to complete its viral replication cycle.

Upon investigation of STIV-infected cells by both scanning and transmission electron microscopy, STIV initiates an unusual cell lysis pathway [15]. One component of this pathway is the formation of unusual pyramid-like structures on the surface of infected cells (Figure 2). These seven-sided projections are believed to be the result of the cell membrane protruding through a disruption in the *Sulfolobus* S-layer [15]. It is thought that, through programmed disruption of these pyramid-like structures, assembled virus particles are released from the cell. This was the first observation of these unusual virus-induced cellular lysis structures, but, subsequently, these same type of structures have also been reported to form in a second totally unrelated virus infection by SIRV2 (*Sulfolobus islandicus* rod-shaped virus 2) [16]. It is interesting that two different viruses with little or no genetic similarity would share similar modes of cell lysis, suggesting that this might be a new lysis mechanism common to many archaeal viruses. Upon closer comparison of the

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**Figure 1** | Genetic map of STIV

The ORFs in grey indicate the viral proteins identified by proteomic analysis of purified virus particles.
SIRV2 and STIV genomes, there is one ORF that shows similarity between the two viruses [17]. SIRV2 ORF p98 and STIV ORF c92 are 55.4% identical at the amino acid level [17]. It is tempting to hypothesize that the gene products of ORFs p98 and c92 play a common role of directing production of cellular pyramid-like lysis structures in each of their respective viral replication cycles.

**Viruses and cellular ESCRT proteins**

The ESCRT machinery is required for eukaryotic endosomal trafficking, viral egress and membrane abscission during cell division [18]. The ESCRT machinery was originally identified in yeast as being necessary for sorting biological molecules into vesicles. The eukaryotic ESCRT machinery consists of four protein complexes: ESCRT-I, -II, -III and -IV. In addition to these protein complexes, several ESCRT-associated proteins are necessary for proper ESCRT function, including the Vps4 AAA+ protein. Several hyperthermophilic crenarchaeota contain homologues for ESCRT-III-like proteins and the Vps4 AAA+ protein that are required for cell division. As opposed to the eukaryotic ESCRT machinery, only ESCRT-III-like proteins have been identified in Archaea. Samson et al. [18] showed that the ESCRT-like machinery from *Sulfolobus* was required for cytokinesis. Using immunostaining techniques, these researchers were able to show a localization of ESCRT-III-like proteins to the midpoints of dividing cells. Furthermore, when cells were transformed with a trans-dominant-negative mutant of the Vps4 ATPase (Walker B mutation), the resultant phenotypes were enlarged cells with elevated DNA content and a large number of ghost cells without DNA [18]. In addition, the deletion of the ESCRT-like components also resulted in *Sulfolobus* cells that did not properly divide.

Viral egress requires a similar membrane fission event as is necessary for cytokinesis. Several eukaryal viruses are known to ‘hijack’ cellular ESCRT machinery in order to facilitate intracellular virus transport and release from the host cell [19–24]. Most of these viruses are RNA viruses, such as HIV-1, ebola virus, HCV (hepatitis C virus) and paramyxovirus PIV-5; however, a handful of DNA viruses are known to use cellular ESCRTs as well (hepatitis B virus, vaccinia virus, and Epstein–Barr virus) [19]. All of the aforementioned viruses are enveloped viruses that are known to use the cellular ESCRT proteins in order to facilitate virus egress [19–23]. In particular, these viruses encode short sequences (L-domains) within their structural proteins that are required for viral egress through the host membrane [19]. The cellular partners for the viral L-domains have been discovered to be ESCRT components [19]. The disruption of cellular ESCRT function or deletion of viral L-domains results in defects in the viral egress pathway illustrating the requirement of ESCRT proteins in the viral replication cycle.

It has been discovered in eukaryotic systems that proteins recruited to mid-cell during cytokinesis are also required in HIV-1 [21] and HCV [23] budding. HIV-1 utilizes a subunit of ESCRT-I and Alix [ALG-2 (apoptosis-linked gene 2)-interacting protein X], an ESCRT-associated protein [21]. However, there was no evidence of ESCRT-I and Alix in the HCV life cycle [23]. Instead, HCV utilizes ESCRT-III and Vps4 [23]. For HIV-1, the deletion of ESCRT components blocks the release of viral particles from infected cells [22]. It is currently believed that, in HCV infections, the ESCRT system may be involved in the intracellular trafficking of viral particles to the membrane [23]. The strategy of utilizing cellular ESCRT proteins for viral budding is not universal. Unlike HIV-1 and HCV, the influenza virus does not rely on cellular ESCRT machinery to exit the host cell [25]. Instead, the influenza virus relies on its own protein, the M2 proton-selective ion channel [25]. There are other examples of viruses not utilizing any cellular proteins in order to exit from the host cell [19].

The previous examples clearly illustrate the requirement for ESCRT components in eukaryotic viral budding, but the question remains whether this is an acquired or conserved role of cellular ESCRT proteins.

**Implications and future work**

We are interested in determining whether the *Sulfolobus* virus STIV uses archaeal cellular ESCRT-like machinery to aid in the assembly and egress of virus particles from infected cells. We have several lines of evidence that would point to this being the case. First, transcriptomics studies have shown the hypothetical ESCRT-III operon in *S. solfataricus* was up-regulated during STIV infection [14]. Secondly, proteomic analysis has found an ESCRT-like protein associated with STIV particles [6]. And finally, it is tempting to speculate that the formation of the STIV-induced pyramid-like lysis structures involves the archaeal cellular ESCRT-like machinery. Structural studies of STIV have already revealed a common ancestry for archael, bacterial and eukaryal viruses. For example, the coat proteins from viruses infecting hosts from the three domains of life (STIV, adenovirus, PBCV1 and PRD1) have nearly identical jellyroll folds [10]. If STIV does utilize *Sulfolobus* ESCRT...
components, it would be the first described case of an archaeal virus ‘hijacking’ cellular ESCRT components for virus release and would strengthen further the evolutionary link between archaeal and eukaryotic viruses.

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**References**


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