Virology 415 (2011) 6–11

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Development of a genetic system for the archaeal virus *Sulfolobus* turreted icosahedral virus (STIV)

Jennifer Fulton Wirth [a,b], Jamie C. Snyder [a,c], Rebecca A. Hochstein [a,b], Alice C. Ortmann [a,c,1], Deborah A. Willits [a,c,2], Trevor Douglas [a,d], Mark J. Young [a,b,c,*]

[a] *Thermal Biology Institute, Montana State University, Bozeman, Montana, USA*
[b] *Department of Microbiology, Montana State University, Bozeman, Montana, USA*
[c] *Plant Sciences and Plant Pathology, Montana State University, Bozeman, Montana, USA*
[d] *Chemistry and Biochemistry, Montana State University, Bozeman, Montana, USA*

ARTICLE INFO

Article history:
Received 1 February 2011
Returned to author for revision
20 February 2011
Accepted 21 March 2011
Available online 15 April 2011

Keywords:
Sulfolobus turreted icosahedral virus (STIV)
Archaea
Infectious clone
Gene disruption
Sulfolobus solfataricus
Yellowstone National Park

ABSTRACT

Our understanding of archaeal viruses has been limited by the lack of genetic systems for examining viral function. We describe the construction of an infectious clone for the archaeal virus *Sulfolobus* turreted icosahedral virus (STIV). STIV was isolated from a high temperature (82 °C) acidic (pH 2.2) hot spring in Yellowstone National Park and replicates in the archaeal model organism *Sulfolobus solfataricus* (Rice et al., 2004). While STIV is one of most studied archaeal viruses, little is known about its replication cycle. The development of an STIV infectious clone allows for directed gene disruptions and detailed genetic analysis of the virus. The utility of the STIV infectious clone was demonstrated by gene disruption of STIV open reading frame (ORF) B116 which resulted in crippled virus replication, while disruption of ORFs A197, C381 and B345 was lethal for virus replication.

Introduction

We have a limited understanding of the viruses that infect cellular hosts belonging to the Archaea. There are five recognized or proposed phyla within the domain Archaea, with the majority of viruses having been isolated from members of the two main phyla, Euryarchaeota and Crenarchaeota. However, to date only a limited number of archaeal viruses have been isolated. There are a total of 49 crenarchaeal and euryarchaeal viral genomes sequenced (Ackermann and Kropinski, 2007; Happonen et al., 2010; Prangishvili et al., 2006; Redder et al., 2009; Snyder et al., 2003). Few of the open reading frames in any of these viruses have significant similarity to known genes in the public databases. Seven new viral families have been created to accommodate this diversity, with more families likely to be added soon. Infectious clones have only been developed for members of the Fuselloviridae (Stedman et al., 1999; Jonuscheit et al., 2003; Berkner and Lipps, 2008) and the spherical euryarchaeal viruses (Porter and Dyall-Smith, 2008).

Sulfolobus solfataricus has emerged as a model organism for the phylum Crenarchaeota. It is an aerobic heterotrophic thermoacidophile (~80 °C and pH 2–4 optimum) that can be easily cultured in the laboratory. Members of the *Sulfolobales* have been detected in high temperature (72–85 °C) acidic (pH 2.0–4.0) hot springs, fumaroles, and mud pots throughout the world (Brock et al., 1972). The genome of *S. solfataricus* strain P2 has been sequenced (She et al., 2001) and a genetic system has been developed for a close relative of this strain (Berkner and Lipps, 2008; Contursi et al., 2003; Schelert et al., 2004; Wagner et al., 2009). Because of the ease of culturing this genus, 15 viruses have been identified that infect *Sulfolobus* spp. (Arnold et al., 2000a,b; Prangishvili et al., 1999; Rice et al., 2004; Schleper et al., 1992; Stedman et al., 2003; Wiedenheft et al., 2004; Xiang et al., 2005).

Sulfolobus turreted icosahedral virus (STIV) was originally isolated from a high temperature (82 °C) acidic (pH 2.2) hot spring in the Rabbit Creek thermal area of Yellowstone National Park, USA. The virus was isolated from an enrichment culture of an uncharacterized *Sulfolobus* species (Rice et al., 2004). The purified virus infects the Italian isolate of *S. solfataricus* strain P2. The virus particle is a 74 nm icosahedron ($T=31$) with turret-like projections extending from its five-fold axes of symmetry (Rice et al., 2004; Khayat et al., 2005). The

* Corresponding author at: 103 Chemistry and Biochemistry Building, Montana State University, Bozeman, Montana, 59717, USA. Fax: +1 406 994 1975.
E-mail address: myoung@montana.edu (M.J. Young).
[1] Current address: University of South Alabama, Mobile, AL 36688-0002, USA.
[2] Current address: Ligocyte Pharmaceuticals, 2155 Analysis Drive, Bozeman, MT 59718, USA.

0042-6822/$ – see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.virol.2011.03.023

virus encapsidates a 17,663 base pair double-stranded circular DNA genome encoding 37 open reading frames. Few of the ORFs have any similarity to known genes in the public databases and thus are difficult to annotate without structural or functional studies (Khayat et al., 2005; Larson et al., 2007a,b; Larson et al., 2006; Maaty et al., 2006; Rice et al., 2004). The replication cycle of STIV is not known, but comparative genomic and structural analysis has provided insights into several STIV gene functions. Proteomic analysis of purified virions has demonstrated that the virion is comprised of nine viral proteins (C557, C381, B345, A223, B164, B130, B109, A78, and A55) and two host proteins (SSO7D and SSO0881) (Maaty et al., 2006). B345 has been determined to be the major capsid protein (MCP) (Rice et al., 2004; Khayat et al., 2005), and its structural analysis has provided evolutionary insights into the relationship between icosahedral viruses from all three domains of life (Khayat et al., 2005). B164 has been shown by homology modeling to likely be a viral ATPase (Maaty et al., 2006). The X-ray crystal structures of three additional STIV proteins, F93, B116 and A197, have been determined. F93 is a winged-helix DNA-binding protein that is a putative transcriptional regulator (Larson et al., 2007a). B116 is a novel DNA binding protein that has been shown to bind DNA in a non-specific manner (Larson et al., 2007b). A197 is a putative glycosyltransferase (Larson et al., 2006).

While STIV is one of the better understood viruses infecting a crenarchaeal host, much remains to be examined. The ability to understand the replication cycle of this virus, the details of its interactions with its host, and the function of its gene products would be greatly facilitated by the development of an infectious clone that allows for the directed manipulation of the STIV genome. There is currently only one other crenarchaeal virus for which there is an infectious clone, *Sulfolobus* spindle-shaped virus (SSV) (Stedman et al., 1999). This paper describes the development of an infectious STIV clone and its utility for probing viral gene function.

Results

Construction of the full-length STIV/TA clone and demonstration of infectivity

A full-length STIV clone was successfully constructed by PCR amplification of three overlapping segments of the native STIV genome and insertion into the *E. coli* pCRII-TOPO-TA plasmid. An *Eco*RI digest of the full-length clone produced the expected banding pattern (Fig. 2A) and DNA sequencing of selected regions of the cloned viral DNA confirmed that the cloned DNA was identical to the native STIV genome. The only exception was the introduction of a new *Bam*HI restriction endonuclease site to facilitate the removal of the pCRII-TOPO-TA vector sequence prior to transfection. The creation of this *Bam*HI site did not result in any altered protein coding capacity within the viral genome.

Transfection of the full-length clone into *S. solfataricus* resulted in the production of both progeny viral particles (Fig. 2B) and genomes (Fig. 2C). Purification of virus from the primary transformed cultures and passage of this virus into early log *S. solfataricus* cells demonstrated that the cloned virus was infectious and produced plaques similar to native virus. Transfection of DNA without prior removal of the *E. coli* vector was not successful. However, the presence of 106 bp of vector sequence in the non-coding region of the STIV genome did not affect infectivity. This indicates that the presence of the entire 3.9 kb of pCRII-TOPO-TA vector sequence prevents viral replication. Likewise, transfection with linear *Bam*HI digested STIV genome was non-infectious. Transformation of the circular cloned viral DNA resulted in an infection cycle that was delayed by 20 hours as compared to native STIV infection, but eventually reached the same magnitude as infection with native virus as estimated by qPCR (Fig. 2C). However, subsequent infection with virus particles obtained from a transfected culture produced a similar timing as native virus. Due to the low plating efficiency a pfu/μg value for transformation efficiency was not determined. Instead, a qPCR assay was used to estimate the number of new viral genomes per mL of media per μg of transformed DNA (Table S2). Transformation efficiency of *S. solfataricus* strain 2-2-12 with the cloned STIV DNA was approximately 400 fold reduced as compared to infection with purified virus (1.5×10^{11} genomes/mL per produced per 1 μg DNA (1.8×10^{10} genomes) vs. 5.7×10^{11} genomes/mL produced per 4.8×10^{8} genomes of virus used as inoculum). In an attempt to assess the host range of STIV, various hosts were tested for infection with native virus and transfection with the cloned STIV DNA. The pattern of successful transfection closely followed the pattern of host competency for infection with native STIV (Table S2). Only *S. solfataricus* supported viral replication.

STIV infectious clone allows for targeted gene alterations

Selected STIV gene disruptions and point mutations were performed to demonstrate the ability of the STIV full-length clone to serve as the basis for a viral genetic system. STIV ORFs A197, B116, B345, and C381 were chosen for gene modification experiments. Disruption of A197, B345, and C381 were lethal to virus replication, with no new viral genomes detected by qPCR. In contrast, transfection with cloned viral DNA harboring a B116 frame shift mutation and a single conserved amino acid substitution within A197 produced infectious virus. These cell cultures were both positive by qPCR for the MCP gene and produced virus particles. The virus isolated from the supernatant of these cultures was infectious when introduced into fresh *S. solfataricus* cells (Fig. 3A). Maintenance of the B116 frame-shift site through multiple passages was confirmed by DNA sequencing. A comparison between the wild type and B116 frame-shift virus infections showed that the B116 frame-shift produced a delayed infection cycle of 24 hours as compared to wild type, but eventually reached similar levels (Fig. 3A). Western blot analysis with B116 antibodies did not detect any B116 protein in the B116 frame-shift transformed cells (Fig. 3B); however, this may reflect our detection limits rather than a true absence of the B116 protein, as genome replication was at a lower level in those samples as compared to wild type. Plaque assay analysis showed that the virus still lysed its host cells, but manifested as a small plaque phenotype. The average plaque size produced by the B116 frame-shift mutant was reduced by ~90% as compared to wild type virus (Fig. 4A, B, C).

Discussion

Construction of an infectious clone of STIV provides a valuable resource for the genetic analysis of this virus and for probing its interaction with its *Sulfolobus* host. The observation that the cloned

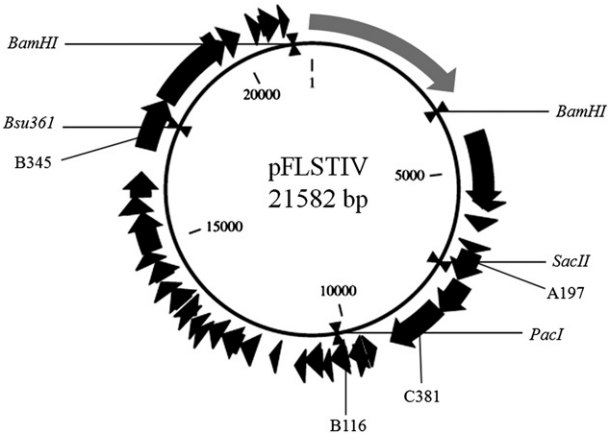

Fig. 1. Genome map of the full-length clone of STIV. STIV ORFs shown in black and the pCRII-TOPO-TA vector shown in gray.