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Structural and Genomic Properties of the Hyperthermophilic Archaeal Virus ATV with an Extracellular Stage of the Reproductive Cycle

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A novel virus, ATV, of the hyperthermophilic archaeal genus *Acidianus* has the unique property of undergoing a major morphological development outside of, and independently of, the host cell. Virions are extruded from host cells as lemon-shaped tail-less particles, after which they develop long tails at each pointed end, at temperatures close to that of the natural habitat, 85 °C. The extracellularly developed tails constitute tubes, which terminate in an anchor-like structure that is not observed in the tail-less particles. A thin filament is located within the tube, which exhibits a periodic structure. Tail development produces a one half reduction in the volume of the virion, concurrent with a slight expansion of the virion surface. The circular, double-stranded DNA genome contains 62,730 bp and is exceptional for a crenarchaeal virus in that it carries four putative transposable elements as well as genes, which previously have been associated only with archaeal self-transmissable plasmids. In total, it encodes 72 predicted proteins, including 11 structural proteins with molecular masses in the range of 12 to 90 kDa. Several of the larger proteins are rich in coiled coil and/or low complexity sequence domains, which are unusual for archaea. One protein, in particular P800, resembles an intermediate filament protein in its structural properties. It is modified in the two-tailed, but not in the tail-less, virion particles and it may contribute to viral tail development. Exceptionally for a crenarchaeal virus, infection with ATV results either in viral replication and subsequent cell lysis or in conversion of the infected cell to a lysogen. The lysogenic cycle involves integration of the viral genome into the host chromosome, probably facilitated by the virus-encoded integrase and this process can be interrupted by different stress factors.

Keywords: virus; archaea; hyperthermophile; genome

Introduction

It is considered that the majority of viruses in aquatic systems constitute head-tailed bacteriophages[1] based mainly on electron microscopic observations of virus-like particles (VLP) in water samples at moderate and low temperatures. Consistent with this inference, most viruses of mesophilic and moderately thermophilic bacteria and archaea, which have been characterised show a strong bias to head-tailed phages[2] (97%). However, recent studies reveal a significantly different and much more complex picture of viral diversity in geothermally heated aquatic habitats above 80 °C. Here viruses with novel and remarkably diverse morphotypes predominate.[3–8] About two dozen of these, all with double-stranded (ds) DNA genomes,[9] have now been isolated and characterised. They infect hyperthermophilic archaea of the genera *Sulfolobus*,

Abbreviations used: ATV, *Acidianus* two-tailed virus; VLP, virus-like particle; p.i., post infection; ORF, open-reading frame; ds, double-stranded.

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Acidianus, Thermoproteus and *Pyrobaculum*, and exhibit a broad spectrum of diverse virion morphotypes many of which have not been encountered amongst known dsDNA viruses from the other domains of life, the Bacteria and Eukarya.[9,10] As for the virion morphotypes, genome structures and sequences also reveal exceptional properties. Comparative genomic analyses clearly indicate that dsDNA viruses of hyperthermophilic archaea are unrelated to any other viruses and have a unique origin, or more likely, multiple origins.[11] To date, they have been classified into six new viral families, including the spindle-shaped *Fuselloviridae*, the filamentous *Lipothrixviridae*, the rod-shaped *Rudiviridae*, the droplet-shaped *Guttaviridae*, the spherical "*Globuloviridae*" and the bottle-shaped "*Ampullaviridae*".[9]

Recently, while systematically investigating the viral diversity of hot springs in Italy, a hyperthermophilic archaeal virus was discovered with a most unusual property. The *Acidianus* two-tailed virus (ATV) underwent a major morphological development outside of the host cell under certain environmental conditions.[12] This active, irreversible biological process, involved the protrusion of tail-like appendices from the virion body and it occurred in the complete absence of the host or any other cell. Moreover, this occurs in the culture medium, in buffer or even in distilled water, as long as the temperature of the environment is above 75 °C. The tail development is fastest at 85 °C, close to the temperature of the natural habitat.

Here, we describe structural and genomic properties of ATV and its relationship with the host cell.

Results

Virus isolation

The virus ATV was purified from the cell-free supernatant of an infected culture of "*Acidianus convivator*" strain AA9. When infected, cells were grown at 75 °C for about five days post infection (p.i.). The purified virus preparation represented a mixture of virions in different stages of extracellular tail development, ranging from tail-less particles to particles with two long tails (Figure 1(a)). In order to purify tail-less virions, the infected host culture was grown at 75 °C for only two days p.i., when almost all

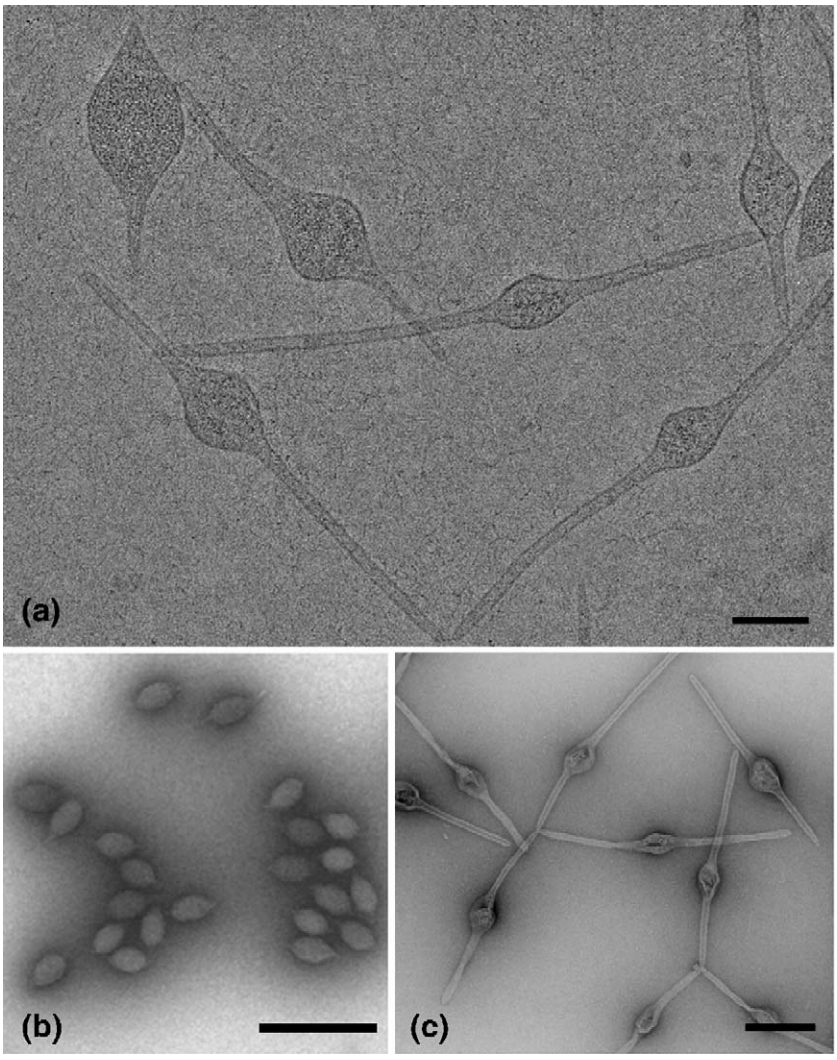

Figure 1. Electron micrographs of purified ATV virions. (a) Cryo-electron microscopy of virions at different stages of tail development. The scale bar represents 100 nm. (b) Transmission electron microscopy (TEM) of negatively stained (2% (w/v) uranyl acetate (pH 4.5)) tail-less virions. The scale bar represents 100 nm. (c) TEM of negatively stained (2% uranyl acetate) two-tailed virions. The scale bar represents 200 nm.