Future VIROLOGY

EDITORIAL

Hard out there: understanding archaeal virus biology



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Each of the three domains of life, Archaea, Bacteria and Eukarya, is associated with a specific virosphere. Despite the fact that archaeal viruses represent only a minute portion of the characterized virosphere, they have recently gained wider attention, mainly due to the unexpected morphological properties of their virions and the unprecedented molecular mechanisms employed throughout their life cycles. Archaeal viruses are currently classified into 15 different families [1,2]. Especially remarkable are the viruses of the hyperthermohilic archaea; these viruses are extremely diverse morphologically and include members with lemon-shaped, droplet-shaped and bottle-shaped virions [1]. Furthermore, the viral genomes encode proteins with little to no significant similarity to proteins in public databases and often possess unique structural folds [3]. Although classical biochemical and genomic studies have yielded important information on the architectures of several hyperthermophilic archaeal viruses, as well as on the functions of some viral proteins, the molecular mechanisms underlying different aspects of the infection cycle remain poorly understood for most of these viruses.

Studies on bacterial and eukaryotic viruses have benefited from the availability of well-established genetic tools that have been developed for the respective hosts and, more generally, from the broad knowledge base on the host biology. This, unfortunately, has not been the case for most of the archaeal virus–host systems. The assays that are considered trivial when thinking about bacterial or eukaryotic viruses (e.g., the plaque test used for virus particle enumeration) present difficulties in the case of hyperthermophilic archaeal viruses. Indeed, the cultivation of hyperthermophilic acidophiles, such as *Sulfolobus*, which, for optimal growth, requires 80°C and pH 2–3, might be challenging. Similarly, live-cell imaging at physiological temperatures, which is widely used to investigate virus–host interaction in eukaryotes, is normally also off the table when dealing with hyperthermophiles. Consequently, the scientific inquiries into the properties of hyperthermophilic archaeal viruses have been, for a long time, limited by the lack of adequate tools.

KEYWORDS
• Archaea • hyperthermophiles • *Sulfolobus* rod-shaped virus 2 • virus evolution • virus–host interactions

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Given all of these difficulties, one might wonder why anyone would bother with studying archaeal viruses in the first place. The major incentives are the following. First, the morphological diversity of hyperthermophilic archaeal viruses is astonishing [1]. Whereas sampling of the bacterial virosphere seems to have reached convergence (i.e., no truly new morphotypes of bacterial viruses have been discovered for decades), virions with unique, previously unseen morphologies are constantly being discovered in the Archaea. It has been suggested that the archaeal virosphere more closely reflects the ancient diversity of viruses on our planet [1]. Consequently, exploration of the archaeal virus diversity provides an exclusive opportunity to learn about the ancient viral architectures that might not have been retained in other cellular domains. Second, the molecular mechanisms underlying virus–host interactions in Archaea combine components that are specific to archaeal viruses with those that are shared with viruses infecting other cellular domains. Thus, in addition to uncovering new Archaea-specific features that are sometimes breathtakingly elegant (as in the case of the recently discovered pyramidal egress structures [4,5]), these studies allow us to better understand the origin and evolution of the mechanisms underlying the infection processes of viruses infecting eukaryotic hosts (see below). Third, due to their ability to withstand harsh environmental conditions, hyperthermophilic archaeal viruses contain considerable appeal for developing various bio- and nano-technological applications. Furthermore, the enzymes encoded by these viruses can be potentially employed for molecular biology applications.

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During the past few years, many modern high-throughput techniques have been adapted for studying archaeal viruses, and new genetic tools have been developed for an increasing number of archaeal hosts and their viruses [6–8]. These newly developed/adapted approaches and genetic tools, in combination with the more classical biochemical techniques, have recently yielded valuable information on the biology of some archaeal viruses. Two hyperthermophilic viruses infecting *Sulfolobus* species have been investigated from different perspectives and served as models for understanding the biology of archaeal viruses. These include *Sulfolobus* turreted icosahedral virus (STIV) and *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2). These two viruses fundamentally differ from each other in virion morphology, genomic content and viral cycle [1]. STIV is a prototype member of the family *Turriviridae*. The STIV virion consists of an icosahedral protein capsid that covers the lipid membrane vesicle enclosing the circular dsDNA genome [9]. Such a virion architecture is commonly found in bacterial and eukaryotic viruses [10]. SIRV2 is the type organism of the family *Rudiviridae*, which comprises viruses with elongated rod-shaped particles containing linear dsDNA genomes [11]. The termini of SIRV2 virions are decorated with terminal protein fibers that mediate the attachment of the viral particles to the pili-like appendages at the host cell surface [12]. Interestingly, despite profound morphological and genomic differences, both STIV and SIRV2 utilize a unique virion release mechanism involving the formation and opening of large pyramidal structures at the surface of the host cell [4,5].

Even though high-throughput approaches generate impressive amounts of data, the comprehensive picture of the viral infection cycle can only be unraveled using a combination of different high-throughput and more classical techniques targeted at particular aspects of the infection cycle and specific viral proteins. Indeed, clues obtained in the course of high-throughput studies have proved to be instrumental for identifying prominent players in the viral life cycles and designing targeted studies in order to understand the functions of these proteins. For example, RNA sequencing analysis of SIRV2-infected cells has revealed that *ORF83a/b* transcripts are dominant, starting within the first minutes of infection and remaining abundant throughout the infection [13], predicting an important role for P83a/b. The x-ray structure of the P83a/b homolog from rudivirus SIRV1, which was solved during the structural genomics project, revealed a helix-turn-helix DNA-binding motif, suggesting that the protein might be involved in the processing of viral DNA [3]. Subsequent yeast two-hybrid analysis has showed that P83a/b interacts with the subunit of the host-encoded PCNA, a processivity factor for DNA polymerase [14]. These results indicate that P83a/b might be responsible for recruiting the PCNA for viral genome replication. Consequently, the complementary results

obtained from different studies illuminated a key role of P83a/b in SIRV2 propagation, providing a framework for further inquiries into the molecular mechanisms of its action.

An important step forward in understanding the biology of archaeal viruses has also been obtained for the example of STIV by the combination of different approaches. In this case, large-scale proteomic analysis of infected cells by 1D and 2D differential gel electrophoresis coupled with protein identification by mass spectrometry and activity-based protein profiling has been used to investigate the interaction between STIV and two *Sulfolobus solfataricus* strains (P2 and P2-2-12) that significantly differ with respect to their susceptibility to STIV [15,16]. In the highly susceptible P2-2-12 strain, only ten cellular proteins were changed in abundance. By contrast, 71 host proteins representing 33 different cellular pathways were affected during the infection of the poorly susceptible P2 strain [15,16], shedding some light on the basis of the different susceptibilities to infection of closely related *Sulfolobus* strains. Most notably, among the highly upregulated proteins were components of the antiviral CRISPR-Cas system and cell division proteins that are homologous to the eukaryotic endosomal sorting complexes required for transport (ESCRT) machinery [17,18], suggesting that the latter proteins play an important role in the STIV infection cycle. In eukaryotes, the ESCRT machinery is employed as the major escape route for many enveloped viruses, including important human pathogens, such as retroviruses, filoviruses, paramyxoviruses and herpesviruses [19]. Importantly, a recent study has confirmed a critical role of the archaeal ESCRT proteins during the late stages of STIV infection, specifically during the maturation of the virion membrane and possibly the opening of pyramidal portals located at the host cell envelope and involved in the release of viral progeny [20].

To conclude, a combination of different high-throughput approaches with more conventional biochemical and microscopic techniques has helped us to uncover the secrets of the enigmatic archaeal viruses. Even though studies on viruses thriving in extreme environments remain challenging, they are also highly rewarding. We have learned a great deal about the inventiveness of these viruses and new surprises are certainly expected in the future. The detailed understanding of archaeal viruses and their interactions with their hosts will enable comparisons with the bacterial and eukaryal virus–host systems, which should eventually reveal the general tendencies underlying the functioning of the virosphere.

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