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Archaeal viruses—novel, diverse and enigmatic

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Recent research has revealed a remarkable diversity of viruses in archaeal-rich environments where spindles, spheres, filaments and rods are common, together with other exceptional morphotypes never recorded previously. Moreover, their double-stranded DNA genomes carry very few genes exhibiting homology to those of bacterial and eukaryal viruses. Studies on viral life cycles are still at a preliminary stage but important insights are being gained especially from microarray analyses of viral transcripts for a few model virus-host systems. Recently, evidence has been presented for some exceptional archaeal-nspecific mechanisms for extra-cellular morphological development of virions and for their cellular extrusion. Here we summarise some of the recent developments in this rapidly developing and exciting research area.

virus morphotypes, diversity and evolution, life cycle, temporal regulation, cellular extrusion mechanism

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1 Historical

Over the past two decades a major revolution has occurred in our understanding of viruses, their evolution and roles in cellular evolution. Until recently, classification of viruses followed, and reinforced, the discredited prokaryote-eukaryote paradigm of living forms that dominated biological thinking throughout much of the 20th century. There were prokaryotic viruses, bacteriophages, primarily exhibiting head-tail morphologies which shared few properties with the more diverse eukaryal viruses. After the discovery of Archaea, the third domain of life, in 1977, this viral perspective initially remained unchanged. In fact, the first attempts to characterise archaeal viruses involved searches for bacteriophage-like particles bearing head-tail morphologies and several examples were found, all associated with haloarchaeal or methanoarchaeal hosts [1]. Simultaneously, however, archaeal virus-like particles (VLPs) were observed that did not conform to this pattern and virions were isolated and characterised primarily from terrestrial hot springs that exhibited a variety of morphotypes, including spindles, spheres, rods, filaments, and other forms, some of which differed radically from bacterial and eukaryal viral morphotypes (Figure 1). These seminal studies, pioneered by Wolfram Zillig and colleagues [2,3], were extensive and highly influential for the subsequent development of the archaeal viral field.

Whereas the early work concentrated on isolating virus-host systems such that the virus could be cultured and investigated in the laboratory, later studies pioneered by David Prangishvili and colleagues focused on enrichingcrenarchaeal viruses, again from terrestrial hot springs, and then testing cultured crenarchaeal strains for compatible hosts of the isolated viruses. This proved to be a rewarding approach and yielded a series of virions with novel morphotypes and exceptional genomes, including the *Acidianus* bottle-shaped virus ABV and the *Acidianus* two-tailed virus ATV (Figures 1 and 2) [4].

In this article we deal with some of the newer developments

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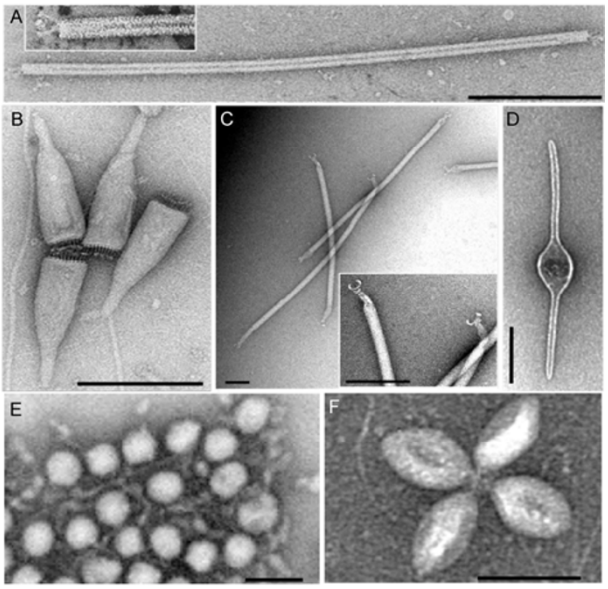

Figure 1 Morphological diversity of archaeal viruses. Electron micrographs of *Sulfolobusislandicus* rudivirus 2, SIRV [5] (A), *Acidianus* bottle-shaped virus, ABV (ampullavirus) [4] (B), *Acidianus* filamentous virus 1, AFV1 (lipothrixvirus) [57] (C), *Acidianus* two-tailed virus ATV (bicaudavirus) [27] (D), *Thermoproteustenax* spherical virus 1 TTSV1 (globulavirus) [20] (E), and *Sulfolobus* spindle-shaped virus 7, SSV7 (fusellovirus) [33] (F). Bars represent 200 nm (A, B, D) and 100 nm (C, E, F).

in the field, focusing especially on recent works on the more extensively studied crenarchaeal viruses and their virus-host relationships. Other recent reviews have covered more general or other specific aspects of archaeal viruses [5–7].

2 Remarkable diversity

While there have been few quantitative analyses of the relative abundance of different virus-like morphotypes in archaeal-rich environments, electron microscopy studies of samples from terrestrial hot springs suggest that spindles, filaments, rods and spheres predominate [8,9], while other morphotypes are much less common. Similarly, spindle-shaped and spherical virus-like particles (VLPs) dominate in hypersaline environments [6,10,11]. Head-tail VLPs are relatively uncommon in archaeal-rich environments, although their proviruses have been detected in several sequenced genomes of halo- and methanoarchaea [6,12].

Virion samples exhibiting a wide variety of morphotypes have been isolated primarily from terrestrial hot springs or hypersaline lakes in many different geographical locations. A number of viruses have been purified and characterisedbased on which several new viral families have been defined, mainly for viruses originating from terrestrial hot springs. Moreover, a few crenarchaeal viruses, together with several haloarchaeal viruses from the euryarchaeal kingdom, remain unclassified. Furthermore, some crenarchaeal and euryarchaeal virions share similar morphotypes, but their genomic contents show little in common. General properties of isolated archaeal viruses are summarised in Table 1. Despite the broad diversity of the archaeal viruses characterised to date, as a group they probably constitute a very biased sample because most of them infect either thermoacidophilic members of the Order *Sulfolobales* or a few haloarchaeal strains.

Most viral genomes fall in the size range of 15–75 kb, and are circular or linear (Table 1). Some linear genomes have free ends whereas others, including the rudiviruses and some lipothrixviruses have covalently closed or otherwise modified ends [13,14]. A few carry base-specific methylations including the haloarchaeal head-tail virus ØCh1 [15] andspindle-shaped viruses His1 and His2 [16],and the

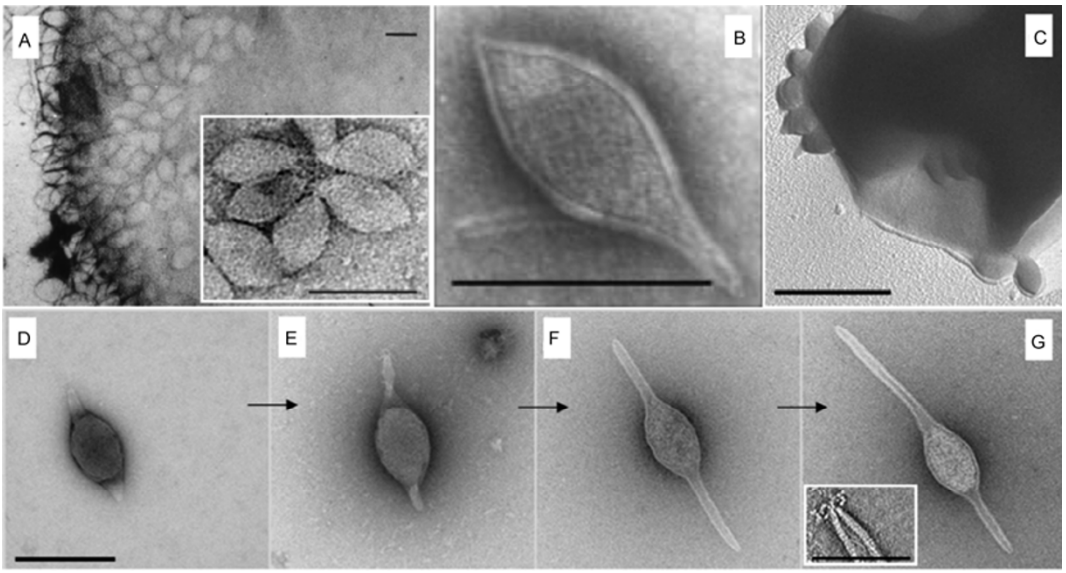

Figure 2 The tailed fusiform viruses. A and B, *Sulfolobus tengchongensis* spindle-shaped virus STSV1 (bars, 200 nm). C–G, Extrusion and extracellular tail development of the bicaudavirus ATV. Bars correspond to 500 nm in C, and 100 nm in D–G (adapted from Xiang *et al.* [18] and Häring *et al.* [27]).

Table 1 General properties of archaeal viruses

Viral family	Morphotype	Genome (ds DNA)	Size range (kb)	Member
Crenarchaea				
Rudiviridae	Rod	Linear	24.6–35.4	SIRV1, SIRV2, ARV1, SRV1
Lipothrixviridae	Filamentous	Linear	21–42	TTV1, SIFV, AFV1, 2, 3, 6, 7, 8, 9
Fuselloviridae	Spindle	Circular	14.7–24.1	SSV1, 2, 4, 5, 6, 7, SSVk1, SSVrh
Bicaudaviridae	Spindle, bipolar tails	Circular	63	ATV
Ampullaviridae	Bottle	linear	23.9	ABV
Globuloviridae	Spherical	linear	21–28.3	PSV, TTSV1
Guttaviridae	Droplet, bearded	Circular, modified	20	SNDV
Unclassifed	Icosahedral	Circular	16.6–17.6	STIV, STIV2
Unclassified	Tailed-fusiform	Circular	75	STSV1
Euryarchaea				
Salterprovirus	Spindle-shaped	Linear	14.5–16.1	His1, His2
Unclassified (haloarchaea)	Spherical	Linear	30.9	SH1
Unclassified (*Pyrococcus*)	Filamentous	Circular	18	PAV1
Mycoviridae(haloarchaea)	Head-tail	Linear	59–78	HF1, HF2, ØH1, ØCh1, Hs1
Siphoviridae (Methanogens, haloarchaea)	Head-tail	Linear	28.7–80	ψM1, ψM2, ψM100, ØF1, ØF3, BJ1

bearded droplet *Sulfolobus* virus SNDV [17], while the tailed-fusiform virus STSV1 encodes three DNA methylating enzymes [18]. The genomes are compactly organised with overlapping genes and minimal intergenic regions, some of which constitute sites for binding of transcriptional regulators. Most viral genes are concentrated on one strand [19,20]. The structural and functional compactness of archaeal viral genomes was reinforced by a transposon mutagenesis study of the spindle-shaped His2 halovirus, which demonstrated that for a total of 34 single transposon insertions in viable mutants, most occurred within the inverted terminal repeats, and only four were located near the downstream ends of three ORFs [21].

Analyses of the emerging archaeal viral genomes revealed surprisingly few sequence matches with genes in the GenBank/EMBL database, and this was extreme for the genomes of the thermoneutrophilic archaeal viruses TTV1, PSV and TTSV1 which showed almost no significant matches (reviewed in [22]). The few homologs that were identified were mainly limited to dUTPases, thymidyl synthases, Holliday junction resolvases, glycosyl transferases and putative transcriptional regulators. These results are summarised for a few crenarchaeal and euryarchaeal viruses in the histogram in Figure 3.

The general lack of insight into protein functions from sequence analyses stimulated the X-ray crystallographic approach to determine structures of viralproteins and to infer their functions from the yielded structures. Several structures have now been resolved, mainly for the smaller capsid and DNA binding proteins but they are accumulating at such a rate, that virion structural models are likely to emerge in the near future, especially for the model icosahedral virus STIV1 [7] and the lipothrixvirus AFV1 [23].

The finding that archaeal viruses, in general, differ radically from bacterial and eukaryal viruses in their genomic properties and often in their morphologies was initially surprising. Then, this result served to reinforce the idea that archaeal viruses are very ancient and may have preceded the separation of the three Domains, such that as each Domain was formed it accommodated a fraction of the existing diverse virus population. Various lines of evidence have added support to this hypothesis. For example, the structure of the coat protein of STIV1 is closely similar to the coat protein structure of the bacterial virus PRD1, and those of the eukaryal viruses PBCV-1 and adenovirus [24]. Moreover, other protein folds, including the double β-barrel, are shared by capsid proteins of unrelated viruses from the three Domains of life [25]. There also appears to be some conservation at a genomic level. A region at one terminus of the linear genome of the archaeal ampullavirus ABV shares both replication genes and limited gene synteny with the corresponding regions of bacteriophage Ø29 and human adenovirus genomes (Figure 4) [26]. Furthermore, gene cassettes of some proviruses in archaeal genomes may encode homologs of proteins involved in virion assembly and maturation of head-tail viruses of archaea and bacteria and of eukaryal herpes viruses [12].

3 Unique bicaudaviruses—a Chinese perspective

One group of archaeal viruses of particular interest are the tailed-fusiform viruses. Their spindle-shaped bodies are attached to long single or bipolar tails (Figures 1 and 2). They were isolated from the Naples region of Italy (*Acidi-*

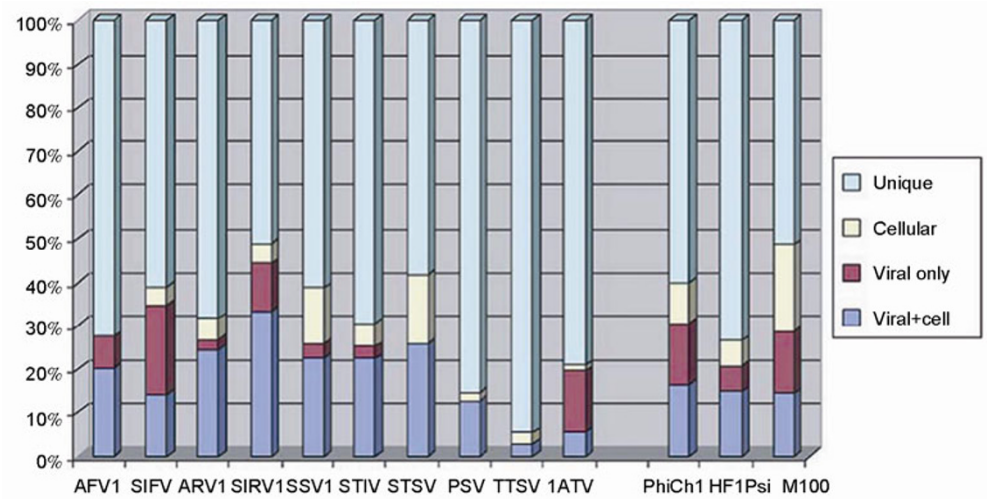

Figure 3 Histogram summarising the low levels of archaeal viral protein matches to public sequence databases for the crenarchaeal viruses (left side) and euryarchaeal viruses (right side). Viral+cell–proteins with homologs in other viruses and cells. Homologous proteins in closely related viruses (including the virus pairs SIRV1-SIRV2 and PSV-TTSV1) are not included (adapted from Prangishvili *et al.* [22]).

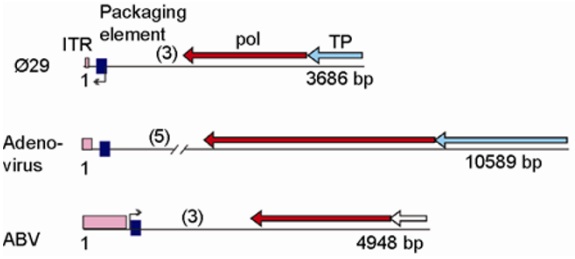

Figure 4 Depiction of similar gene organizations at the left ends of the linear genomes of bacteriophage phi29 (Acc. No. V01121), eukaryal adenovirus type 5 (Acc. No. AC000008) and the archaeal ABV virus, where inverted terminal repeats (ITRs) of lengths 6, 103 and 590 bp, respectively, are represented by pink boxes. Genes encoding the polymerase (pol) and terminal protein (TP) are denoted by red and cyan arrows, respectively, while ORF163 upstream of the putative polymerase in ABV is shown by an empty arrow. The blue box denotes the genomic region indispensable for packaging in phage phi29 and adenovirus. Transcription directions (predicted for ABV) are shown by small arrows. The numbers of ORFs between the packaging elements and the protein-primed polymerase genes are given in brackets (adapted from Peng *et al.* [26]).

anus two-tailed virus, ATV) or the Tengchong region of South-western China (*Sulfolobus tengchongensis* spindle-shaped virus, STSV1) [4,18]. Both viruses carry double stranded DNA genomes that are relatively large for archaeal viruses, 62–75 kb, and they share several homologous genes which are distantly related, including the genes for the major coat protein. They also encode several proteins which yield matches in public sequence databases including, for STSV1, a range of proteins involved in DNA modification and nucleotide metabolism [18]. They have been classified tentatively into the family *Bicaudaviridae* in the GenBank virus database. However, STSV1 probably needs reclassification because the two viruses exhibit major differences in their properties. ATV virions are exceptional in that spindle-form virions can grow bipolar tails independently of the host cell at the optimal temperature for cellular growth (about 80 degrees for the host *A. convivator*) (Figure 2) [27,28]. STSV1 virions do not appear to undergo a similar extracellular development of the single tail [18]. Intriguingly, while STSV1 exhibits one major protein component in its coat proteins, ATV virions contain several major components.Therefore, it is likely that at least some of the major ATV-specific virion proteins could be involved in promoting the extracellular tail development.

4 Transcriptional properties of model crenarchaeal viruses

Recent work using a variety of approaches including Northern blotting and microarray analyses, in combination with genomic and structural studies, have yielded important insights into viral gene function and transcriptional regulation. Most of this work has been done on the model crenarchaeal viruses SSV1, STIV1/2 and SIRV1/SIRV2 all of which infect *Sulfolobus* strains and these results will serve as a good basis for future studies.

4.1 Fuselloviruses SSV1/2

Fusellovirus SSV1 can occur in either an integrated or in a free form with a low copy number of 3 to 4 per cell, both of which are present in uninduced cells. Northern analysis of RNAs prepared from uninduced host cells detects nine transcripts from SSV1 (T1–T9) while an additional transcript T-ind appears only after UV induction [30]. UV irradiation induces viral replication,leading to a high level of virion production (up to 100 per cell) [29]. A recent microarray study has also revealed an additional transcript, Tx, from ORF C124 (Figure 5A) [31]. All transcripts, except

T-ind, are produced constitutively in uninduced cultures [30,31]. However, their expression is regulated temporally, the level of T-ind transcript is elevated greatly 1 h after UV treatment and persists for 4 h. After 3 h, genes upstream from T5 and T6 are expressed and full-length transcripts are induced 2 h later. T9, the last of the early transcripts, appears after 5 h, just before the onset of viral replication. Late transcripts T1/2, T3, and T4 are observed 6 h after UV treatment (Figure 5A) [31].

Microarray results correlate well with the predicted functions of the viral gene products and with the genomic properties of eight other characterised fuselloviruses [32,33]. However, only SSV1 reacts strongly to UV irradiation, exclusively producing T-ind and, almost exclusively, T5 (Figure 5A) [33]. Thus, the T5 gene products are considered to regu- late the UV-induced viral replication, and some proteins encoded by T5 and T6 show features typical of transcriptional factors or other regulatory proteins. For example, crystal structures have revealed that F93 and F112 are winged-helix DNA binding proteins [34,35], E51 and C80 are CopG-like regulatory proteins; while A79, C102a and B129 carry zinc finger motifs all of which are indicative of DNA binding. The last early transcript T9 is produced from six of the 13 core fuselloviral genes [33] and the high sequence conservation and timing of transcription strongly implicate these gene products in viral replication.

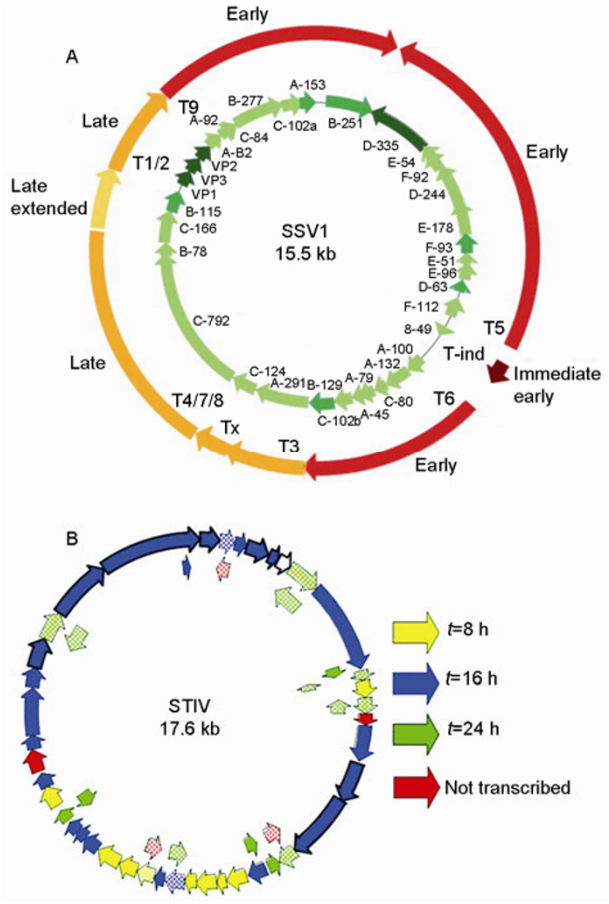

Figure 5 Transcriptome analyses of model *Sulfolobus* fusellovirus SSV1 and icosahedral virus STIV. A, Genome map of SSV1 showing the results of transcript mapping and microarray analyses. Transcripts are categorized according to their time of appearance and (putative) functional roles, into immediate early, early, late and extended late. Genes are colour-coded; dark green - known functions, medium green-predicted functions or light green-unknown functions (adapted from Frols *et al.* [31]). B, Genome map of STIV. Arrows with filled colours correspond to protein genes and the thickly outlined arrows indicate ORFs that constitute virion proteins. Genes are colour-coded according to the time when their transcripts were first expressed. All transcript levels peaked at 24 h p.i. The cross-hatched arrows represent intergenic regions (adapted from Ortmann *et al.* [38]).

Each set of transcripts T1+T2 and T4+T7+T8 initiates from a single promoter and within each set, transcripts differ in length, reflecting the occurrence of some internal regulation. The transcript from C166 and B115 is the last to be significantly up-regulated, 8.5 h after UV irradiation. Thus, T4 appeared about 6 h after UV treatment, while T7 or T8 were up-regulated more than 2 h later [31]. B115 is predicted to be a transcriptional repressor that down-regulates early genes towards the end of the viral cycle [29].

Transcription of a few host genes was also activated on UV irradiation, and the gene products probably contribute to T-ind induction [31]. Of the six host genes linked to the induction of T5 and T6, only SSO1210 was significantly down-regulated and was considered to repress T5 and T6 transcription, while induction of T-ind leads to removal of the repression. Compatible with this hypothesis, sequence similarity (35%/70% identity/similarity) occurs between the C-terminal half of the T-ind protein (B49) and the N-terminal region of SSO1210 such that the two proteins may compete for binding sites at the T5 and T6 promoters and regulate transcription.

Induction of virus replication has also been demonstrated for SSV2, another fusellovirusisolated from an Icelandic hot spring. In contrast to the UV induction of SSV1, SSV2 replication is not to be elevated by an environmental stimulus. Instead, the inductionoccurs most likely as a result of an unknown physiological signal specifically synthesized at a late growth phase of host cells. This virus coexists with the virus satellite pSSVxat in a low copy number in *Sulfolobus islandicus* REY15/4 cells at an early exponential growth phase [32,54]. However, when host cells reacha late exponential growth phase, viral DNA synthesis is strongly induced. In fact, the inductionof virus replicationis concurrent with growth cessation of host cells. The copy number of SSV2 increased toabout 50-fold within 4 h of induction [36]. Furthermore, Northern blotting was employed to study the regulation of gene expression for pSSVx. Initial attempts identified negative regulators including a few species of antisense RNAs [37] but positive regulators responsible for the observed replication induction remain to be revealed.

Clearly, the SSV2/SSVx system represents another exciting viral system for studying archaeal host-virus interactions.

4.2 Turreted icosahedral STIV1

STIV1 propagates in Sulfolobus *solfataricus* strains with a slow life cycle of about 38 h [38]. Viral transcripts are first detected 8 h post infection (p.i.) when nine viral genes, which are likely to be important for initial infection, are expressed (Figure 5B). After 16 h, most viral genes are expressed and, on average, are 12-fold up-regulated (Figure 5B). After 24 h, several genes, including F93, F75, D66, C63 and B66a, and a few intergenic regions, show little or no transcription [38].

A total of 177 host genes were estimated to be differentially expressed during STIV1 infection, with 124 up-regulated, mostly detected between 24 and 32 h, and 53 down-regulated, mainly at 32 h p.i. Of the 41 genes that were up-regulated 4-fold or more many are associated with DNA replication, including *cdc6-1* and *cdc6-3*, or with transcription. Down-regulated genes tend to be associated with production and conversion of energy, lipid metabolism, and transport and metabolism of amino acids and carbohydrates [38]. Therefore, the up-regulated host gene products appear to facilitate viral replication while the down- regulated genes probably reflect a cellular response to approaching cell lysis.

4.3 Rudiviruses SIRV1/2

Transcription from the closely related rudiviruses SIRV1 and SIRV2 was studied using Northern blotting and RT-PCR analyses. A simple transcription pattern was observed, with few genes showing temporal regulation [39]. Most genes were transcribed 30 min p.i. with stronger signals 1 h p.i. A few late transcripts include monocistronic transcripts of ORF399 (near the left ITR), ORF134 (coat protein), ORF55 immediately downstream of ORF134 and a few SIRV1 ORFs close to the right ITR [39]. Start sites of SIRV1 transcripts were mapped by primer extension and subsequent analyses of the promoter regions revealed a trinucleotide GTC motif immediately downstream from archaeal TATA boxes for several genes which could be a recognition site for virus-specific transcription factors. Interestingly, a similar motif GAC proceeded many TATA boxes in the *Acidianus* rudivirus ARV1 genome.

A host-encoded transcription factor, Sta1, was isolated from *S. islandicus* by affinity chromatography using promoter sequences from 3 SIRV1 late genes, ORFs 56, 134 and 399 [40]. The 14 kD Sta1 binds within the core promoter and about 30 bp upstream and, *in vitro*, it stimulates transcription especially at low concentrations of the transcription factors TBP or TFB [40].

SIRV1 ORF56 (gp08), termed SvtR (*Sulfolobus* virus transcription regulator), has been studied structurally and functionally [41]. The structure of the 6.6 kD protein, determined by NMR, consists of an RHH domain between residues 13 and 56 with a disordered N-terminus. The structure resembles that of bacterial RHH proteins despite the very low sequence similarity. Four target sites of SvtR on SIRV1 viral DNA were identified. These include the promoter regions of the SvtR gene, an operon starting from the left ITR (ORFs 90a, 102, 76 and 105), and an operon starting from the right ITR (ORFs 90b, 75, 98 and 252) and the minor virion component ORF1070 [41].

In contrast to SvtR, Sta1 stimulates transcription from SvtR promoters [40]. Both transcription factors can bind to the promoter independently of TBP and TFB, with Sta1 possibly occupying the sequence upstream and SvtR the sequence downstream from the archaeal TATA box, although it remains unclear whether and how they interact functionally.

Unlike the response of SSV1 to UV irradiation, STIV1 and the rudiviruses show little or a very moderate level of temporal regulation. This could either reflect an intrinsic characteristic ofarchaeal viruses orthegeneral lack of their synchronizedinfection. Sincetemporal regulation of gene expression is a common feature for bacterial and eukaryal lytic viruses, it is unusual that STIV and SIRV1/SIRV2, which are exceptional archaeal lytic viruses, do not adopt the same strategy. Conducting similar researches on other archaeal viruses will reveal how widespread this phenomenon is amongst archaeal viruses and what is the advantage of using different strategy to regulate the genome expression of archaeal viruses.

5 Virion packaging and extrusion

The first attempts to view virion assembly *in vivo* were performed on the turreted icosahedral lytic virus STIV1 using electron microscopy [42]. In near synchronously infected cells, mature virions appeared about 24 h p.i, together with immature virus particles lacking dense cores. The lipid membrane of each virus particle formed initially as a rounded crystalline region and the protein shell, composed of the 38 kD major capsid protein either assembled around it or coassembled with the lipids. After formation of the lipid-protein vesicle, turreted structures were formed and inserted into the lipid membrane. The product is an empty, thick-shelled, rounded particle, with a circular inner membrane, devoid of nucleic acid (Figure 6). After packaging of the genome, possibly employing a similar mechanism to bacteriophage PRD1, particles appear more angular with thinner shells characteristic of mature particles (Figure 6) [42]. Simultaneously, pyramidal structures form on the cell surface, in regions devoid of S-layer proteins, through which the virions were inferred to extrude from the cells (Figure 6) [42].

Many other crenarchaeal viruses, including most fusello-

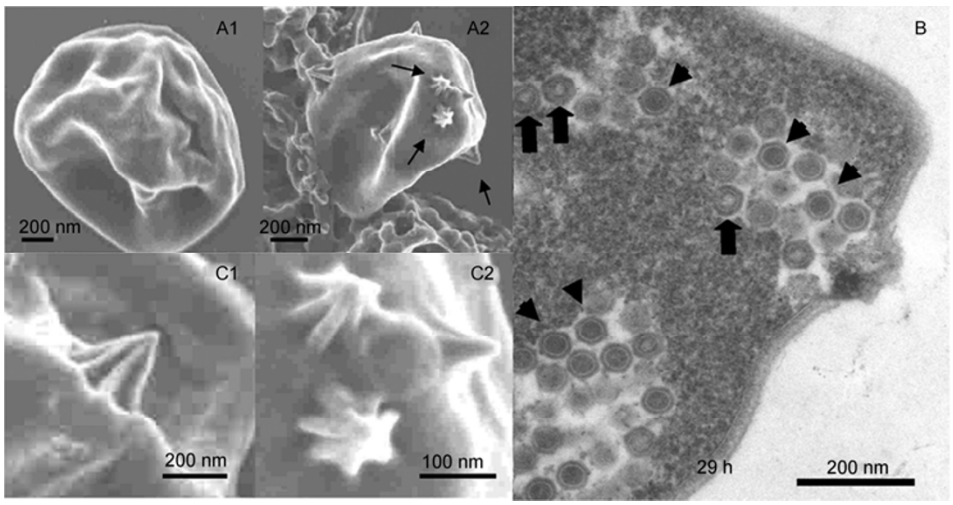

Figure 6 Electron micrographs of S. *solfataricus* cells at different stages of infection with STIV1. A, Non-infected cells (A1) and infected cells (A2) displaying membrane protrusions (thin arrows). Pyramid-like structures from STIV1-infected cells (C1 and C2). B, Mixed populations of immature virus particles lacking dense interior cores (thick arrows) and mature virus particles with dense interior cores (arrowheads). Turret-like projections are present in both mature and immature particles (thin arrows). C, Pyramid-like structures from STIV1-infected cells (C1 and C2). Scale bar sizes are indicated (adapted from Brumfield *et al.* [42]).

viruses, rudiviruses, lipothrixviruses and several haloviruses have been considered to be non-lytic, existing in a carrier state in host cells. For some viruses, the evidence for this has been a lack of optical density decrease and absence of cellular debris in infected *Sulfolobus* cultures [43]. However, a recent study of SIRV2 infection of an S. *islandicus* strain, revealed that colony forming unit (CFU) values dropped 1000-fold 6 h p.i., and flow cytometry and dot hybridization analyses of infected cultures revealed extensive chromosome degradation and enhanced SIRV2 replication, 4 and 8 h p.i., with virus release after 8–10 h, and a burst size of 20–40 virions per cell [44]. Electron microscopy studies of the infected cells revealed dense aggregates of aligned rod-shaped virions, and multiple pyramidal protrusions were observed on the cell surface, similar to those of the STIV1 infected cells, again lacking S-layer proteins on the cell envelope (Figure 7). After release of the virions (about 26 h p.i.), almost all cells were perforated and empty, exhibiting a spherical form, different from the irregular coccoid shape of uninfected cells (Figure 7) [44]. All these observations suggest that host cells die as a consequence of the specific mechanisms orchestrated by the archaeal viruses. They also show that the same extrusion mechanism can be exploited by different viruses, at least for the genus *Sulfolobus*.

The results serve to reinforce the recent re-evaluation of relationships between viruses and their host cells, and the concept of a cell developing into a virion factory. This concept was fuelled by the discovery of the giant eukaryal mimivirus and mamavirus from amoeba, where highly complex virion factories were formed intracellularly, comparable in size to host nuclei and surrounded by membranes deriving from the endoplasmic reticulum [45]. An analo-

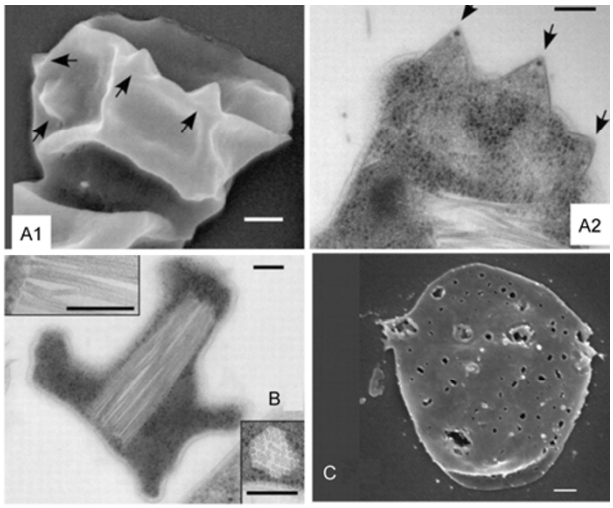

Figure 7 A, Electron micrographs of SIRV2-infected S. *islandicus* cells showing pyramid structures in A1 and A2, indicated by arrows. B, Intracellular virion aggregates, sectioned according to a parallel (upper) or perpendicular (lower) plane. C, Visualisation of perforations in the cell membrane through which viruses have extruded. Scale bars, 200 nm (adapted from Bize *et al.* [44]).

gous process occurs in bacteria, and as indicated above for archaea where infected cells become virion factories as the cellular genome and proteins are degraded and utilised by propagating viruses, at which point the host can no longer be considered a cellular organism.

Despite the complex virion factories observed for STIV1 and SIRV2, most studies on the crenarchaeal and haloviruses suggest that persistent and stable viral infections are common and that viruses extrude from cells without causing lysis (e.g., [4,6,43]). Moreover, this view of continual viral release, without accompanying lysis, is consistent with ar-

chaea carrying a cell membrane coated by a thin glycoprotein surface layer in contrast to the rigid peptidoglycan cells walls of bacteria.

6 Viral evolutionary mechanisms

A puzzling feature of some families of archaeal viruses and plasmids is that they often exhibit regions of highly similar, or even identical, sequences which vary in their genome positions and do not always encode "core" proteins. This has been observed for the crenarchaeal fuselloviruses [32,33,46], the *Sulfolobus* conjugative plasmids [47], and also for the head-tail haloarchaeal viruses HF1 and HF2 [48]. Recently, a hypothesis was proposed to explain this phenomenon for fuselloviruses, which may extend to other archaeal integrative genetic elements and could have more general implications for the evolution of archaeal viruses and plasmids [33]. It was proposed that when different fuselloviruses integrate consecutively into the same tRNA gene, they generate tandem integrated viruses which, if sufficiently similar in sequence, could recombine homologously within the host chromosome to generate one or more hybrid viruses which is then released as a variant virus.

Such a mechanism would provide a rationale for the widespread occurrence of the archaeal-specific integrase which partitions on chromosomal integration. Partitioning of integrase genes favours chromosomal entrapment of the virus, or plasmids, when cells are cured of the free genetic element [49,50] and this, in turn, increases the probability of a double viral integration event occurring at a given tRNA with subsequent formation of novel viral variants, which may then be able to overcome the host immune or other defence systems [33].

Another mechanism involving exchange of genetic modules which may also influence viral evolution has been demonstrated recently for some archaeal viruses andwas characterised earlier for some bacteriophages. This involves gene cassettes encoding a specific viral structural or functional apparatus recombining homologously or non-homologously between viruses [51]. Inter-viral recombination was inferred for the linear ds DNA genomes of non-integrating lipothrixviruses. A 10 kb region of the beta-lipothrixvirus AFV7 had apparently undergone exchange with a 13 kb region of a delta-lipothrixvirus similar to AFV2. Moreover, the corresponding region of another beta-lipothrixvirus SIFV had also apparently been exchanged [52]. It was inferred that the recombined region carries a cluster of genes encoding proteins involved in the formation of terminal tail structures of the filamentous virion, since the genes yield weak sequence matches with membrane proteins, secretion adhesion proteins and tail fibre structures [52]. It is likely that evidence for such mechanisms will accumulate as more viruses are sequenced.

7 Viruses, viral satellites and plasmids

Archaeal viruses and plasmids have evolved complex relationships both as dependents and antagonists and, moreover, it is probable that in extreme cases, they are interchangeable by gaining or losing genes encoding the encapsulating apparatus [53]. An example of a dependent interaction is provided by the relationship between fuselloviruses and pRN plasmids (Table 2). To date, about ten pRN plasmids have been characterized, two of which, pSSVx and pSSVi, can behave as satellite fuselloviruses [54,55], while pXZ1 coexists intracellularly with a fusellovirus [46] (Table 2). pSSVx was isolated together with fusellovirus SSV2 from the native host S. *islandicus* REY15/4 [54] while pSSVi was detected in a *S. solfataricus* P2 lab strain after infection with SSV2 DNA [55]. Both plasmids were packaged into virus-like particles and they spread through *Sulfolobus* cultures in the presence of the helper fusellovirus. Two homologous genes shared by SSV2 and pSSVx were implicated in packaging of the plasmids into capsids [54]. However, since the genes were absent from pSSVi, it is likely that plasmid DNA elements are recognized by the viral packaging apparatus. Another plasmid, pXZ1, coexists stably with the fusellovirus SSV4 in S. *islandicus* ARN3 [46] but it was not packaged and did not spread with the virus in *Sulfolobus* cultures.

There is also some interplay between the viral and plasmid integrase genes. An integrase gene in pXZ1, which is likely to have derived from SSV4 [51], enables the plasmid to integrate into a different host tRNA gene from the SSV4. pSSVi, but not pSSVx, also carries an integrase gene [55] (Table 2). Thus, pRN plasmids can exploit the fuselloviral packaging apparatus and/or integrase gene to enhance their possibilities for survival.

Table 2 Interactions between *Sulfolobus* fuselloviruses and pRN family plasmids

Fusello-virus	Plasmid	SSV-dependent plasmid packaging	Partitioning integrase gene in plasmid	Host	Reference
SSV2	pSSVx	Yes	No	S. *islandicus* strain REY15/4	[32,54]
SSV2	pSSVi	Yes	Yes	S. *solfataricus* strain P2	[55]
SSV4	pXZ1	No	Yes	S. *islandicus* strain ARN3/6	[46]

There are also examples of competition between archaeal viruses and plasmids. Thus, the conjugative plasmid pAH1 resides in both integrated and free forms in *Acidianushospitalis* W1 but infection with the lipothrixvirus AFV1 leads to a loss of the circular form of pAH1 concurrent with an increase in intracellular levels of AFV1 DNA. It was inferred that AFV1 inhibited plasmid replication because no pAH1 degradation was observed [56,57]. Viral inhibition of plasmid propagation also provides a rationale for why CRISPR clusters of the archaeal immune system (see Section 9) are present in some *Sulfolobus* conjugative plasmids. Both pNOB8 and pKEF9 carry CRISPR clusters, and pKEF9 carries a spacer matching very closely to a sequence of the *Sulfolobus* rudivirus SIRV1 [47,58]. The processed plasmid spacer RNA could potentially target and inactivate the virus intracellularly.

8 Viruses and chromosomal evolution

With the recent availability of large numbers of chromosome sequences, it is becoming increasingly clear that viruses, in general, have played a major role in the evolution of host genomes. Many eukaryal genomes are especially rich in integrated retroviruses and repetitive elements of retroviral origin constituting, for example, at least 42% of the human genome sequence, and it has been argued cogently that such retroviral elements generate genetic novelty and drive evolution [59]. There is increasingly strong evidence that a related process may provide increased genetic novelty in archaea and bacteria. Although no archaeal RNA viruses have yet been discovered, and few have been characterised for bacteria, a rapidly increasing number of genes and other sequences of viral DNA origin have been located in archaeal and bacterial genomes, either by direct comparison with sequenced viruses and other genetic elements [50,60], or by using bioinformatical approaches, such as Hidden Markov Model-based strategies to identify clusters of genes with atypical sequence compositions (CAGs) [61]. For archaea, the process of entrapment of genetic elements in host chromosomes is facilitated by the archaeal-specific process, where integrase genes can partition on integration such that an intact integrase protein, required for genome excision, cannot be expressed [49,50,60,62]. Encaptured elements are sometimes recognisable as intact elements, some with genes inactivated by transposable elements, but more often they are only detectable as fragments carrying a few genes, often interrupted or otherwise degenerated, suggesting that there is some selective retention of certain genes and loss of others [60,63]. For example, a cluster of genes almost identical in sequence to two regions of the *Acidianus* two-tailed virus ATV, is maintained in the integrated genetic element XQ2 in the chromosome of *S. solfataricus* P2, where all the gene products are of unknown function [30,49]. Hidden Markov model analyses have suggested that such genes of viral (or plasmid) origin contribute disproportionally to the genes of unknown function in archaeal chromosomes [61].

9 CRISPR adaptive immune systems

Together with the physically linked and highly disparate *cas* genes,CRISPR arrays provide the basis for immune systems primarily directed against viruses and conjugative plasmids in almost all characterised archaea and in many bacteria [64,65]. The CRISPR arrays consist of direct repeats, separated by spacer sequences of regular length, which have been excised from invading genetic elements and inserted adjacent to a leader sequence from which they are transcribed and processed into small crRNAs (reviewed in [64,65]). These crRNAs can then generate crRNA-protein interference complexes that can target and degrade invading elements carrying the same, or a closely similar sequence [66,67]. The CRISPR loci appear to be fairly stable structures, rarely undergoing integration or transposition events, but limiting their own sizes by periodically undergoing internal deletions [68,69]. Thus, the inserted spacer DNA appears to have little long-term influence on host genome contents except, indirectly, by preventing cellular propagation of viruses that might integrate into the host genome. The CRISPR-based systems may, however, strongly influence the evolution of the invading viruses. Experimental evidence suggests that genomes of the rudiviruses and lipothrixviruses may have developed a specific strategy for altering their gene compositions to avoid targeting by the immune systems [70,71].

10 Future perspectives

Archaeal virus biology is entering a productive and exciting phase. Considerable attention, to date, has focused on several thermoacidophilic and a few haloarchaeal viruses in determining their morphological and genomic properties, and this has yielded many new insights into these novel and diverse virus types. However, other environments remain almost unexamined, including those containing thermoneutrophilic and alkaliphilic archaea, as well as mesophilic crenarchaea and methanogens, although the few published examples suggest they will extend the present range of viruses considerably [13]. Enigmatic remain both the evolutionary relationships between the crenarchaeal and haloarchaeal viruses which despite often exhibiting similar morphotypes, show very limited similarities at a genomic level, as well as the evolutionary relationships between the archaeal viruses and those of bacteria and eukarya in general. These puzzles will be addressed more rigorously as sequences of more archaeal viral genomes are determined and virion structural properties are unravelled.

Importantly, as described above, a few robust model viral-host systems have now been established in a few laboratories and basic questions concerning the viral life cycles can be addressed and answered. In particular, one can begin to identify cell receptors, and examine mechanisms of cellular entry, as well as investigating viral DNA replication and gene regulation mechanisms in detail, and the viral and host components that participate in virion assembly and cellular extrusion. The next few years should provide some intriguing insights and exciting developments in this rapidly developing and challenging research area.

11 Afterword

Since this review was completed there have been a number of important developments within the archaeal viral field. A novel short rod-shaped virus, APBV1, was characterised from the thermoneutrophile *Aeropyrum pernix* which carries an exceptionally small genome of 5278 bp and has been classified into a new family the *Clavaviridae* [72]. Several seminal metagenomic studies have been performed examining the viral contents of extreme environments and attempting to link archaeal viral genome sequences to specific virion morphotypes both in terrestrial hotsprings [73,74] and in a hypersaline environments [75,76]. In the near future such studies, particularly when exploiting high throughput sequencing techniques, should provide many important breakthroughs. Furthermore, the first virion carrying a single-stranded DNA genome was characterised, infecting a haloarchaeon [77]. The physiological induction of the *Sulfolobus*SSV2/pSSVx helper-satellite virus system has been further characterised and novel mechanisms for transcriptional termination were unravelled and a positive regulator implicated in virus induction was characterised [78,79]. There have been newer developments in characterising the virion release mechanisms for STIV [80,81] and SIRV2 [82,83] and evidence was provided for a co-chaperone activity of two ATV virion proteins and it was proposed that they played a role in facilitating the extracellular tail development [84]. A putative protein receptor was also characterised for this virus in the membrane of *S. solfataricus* [85]. Finally, rapid progress has been made in understanding the disparate interference mechanisms of archaeal CRISPR-based immune systems (reviewed in [86]).

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