The LUCA and its complex virome

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Abstract | The last universal cellular ancestor (LUCA) is the most recent population of organisms from which all cellular life on Earth descends. The reconstruction of the genome and phenotype of the LUCA is a major challenge in evolutionary biology. Given that all life forms are associated with viruses and/or other mobile genetic elements, there is no doubt that the LUCA was a host to viruses. Here, by projecting back in time using the extant distribution of viruses across the two primary domains of life, bacteria and archaea, and tracing the evolutionary histories of some key virus genes, we attempt a reconstruction of the LUCA virome. Even a conservative version of this reconstruction suggests a remarkably complex virome that already included the main groups of extant viruses of bacteria and archaea. We further present evidence of extensive virus evolution antedating the LUCA. The presence of a highly complex virome implies the substantial genomic and pan-genomic complexity of the LUCA itself.

Viruses and other mobile genetic elements (MGEs) are involved in parasitic or symbiotic relationships with all cellular life forms[1–5], and theoretical models indicate that the emergence of such selfish elements is an intrinsic feature of replicator systems[6–11]. Thus, genetic parasites must have been inalienable components of life from its very beginnings. Unlike cellular life forms, viruses employ all existing types of nucleic acids as replicating genomes packaged into virions. This diversity of the replication and expression strategies has been captured in a systematic form in the 'Baltimore classification' of viruses[12]. Recently, we undertook a comprehensive reappraisal of the findings of virus phylogenomics to assess the evolutionary status of each of the Baltimore classification groups[13]. This synthesis culminated in the identification of four realms (the highest rank in virus taxonomy) of viruses that are monophyletic with respect to their core gene sets and partially overlap with the Baltimore classification: *Riboviria*, *Monodnaviria*, *Duplodnaviria* and *Varidnaviria*. *Riboviria* includes viruses with positive-sense, negative-sense and double-stranded RNA (dsRNA) genomes as well as reverse-transcribing viruses with RNA and DNA genomes. Members of this realm are unified by the homologous RNA-dependent RNA polymerases (RdRPs) and reverse transcriptases (RTs). *Monodnaviria* includes single-stranded DNA (ssDNA) viruses together with small double-stranded DNA (dsDNA) viruses (papillomaviruses and polyomaviruses) that are unified by the distinct endonuclease (or its inactivated derivative) involved in the initiation of genome replication. *Duplodnaviria* include tailed dsDNA bacteriophages and archaeal viruses along with animal herpesviruses that are unified by the distinct morphogenetic module consisting of HK97-fold major capsid proteins (MCPs), homologous genome packaging ATPases-nucleases (terminases), portal proteins and capsid maturation proteases. *Varidnaviria* is an enormously diverse assemblage of viruses infecting bacteria, archaea and eukaryotes that are unified by the vertical jelly-roll MCPs (most groups possess double jelly-roll (DJR) MCPs but some have a single jelly-roll (SJR) domain that is the likely ancestral form) along with a distinct type of genome packaging ATPase present in most constituent groups. This megataxonomy of viruses has been recently formally adopted by the International Committee for the Taxonomy of Viruses[14,15]. Apart from the four monophyletic realms, several groups of viruses remain unaffiliated in the emergent megataxonomy, most notably the diverse dsDNA viruses of hyperthermophilic archaea that form several distinct, seemingly unrelated groups[16–18].

In another recent synthesis, we examined the origins of the replication and structural modules of viruses and posited a 'chimeric' scenario of virus evolution[19]. Under this model, the replication machineries of each of the four realms derive from the primordial pool of genetic elements, whereas the major virion structural proteins were acquired from cellular hosts at different stages of evolution giving rise to bona fide viruses.

In this Perspective article, we combine this recent work with observations on the host ranges of viruses in each of the four realms, along with deeper reconstructions of virus evolution, to tentatively infer the composition of the virome of the last universal cellular ancestor (LUCA; also referred to as the last universal common ancestor of cellular organisms).

The LUCA

Evidently, to make any meaningful inferences regarding the viruses that infected the LUCA, we must have at least a general notion of the characteristics of this ancestral life form. Considerable efforts have been undertaken over the years to deduce the genetic composition and biological features of the LUCA from comparative genome analyses combined with biological reasoning[20–22]. These inferences are challenged by the complex evolutionary histories of most genes (with partial exception for the core components of the translation and transcription systems) that involved extensive horizontal transfer and non-orthologous gene displacement[23–25]. Nevertheless, on the strength of combined evidence, it appears likely that the LUCA was a prokaryote-like organism (that is, like bacteria or archaea) of considerable genomic and organizational complexity[20,26–28]. Formal reconstructions of the ancestral gene repertoires based on maximum parsimony and maximum likelihood approaches assign several hundred genes to the LUCA that are responsible for most of the core processes characteristic of prokaryotic cells[22,27,29], perhaps making it comparable to the simplest extant free-living bacteria and archaea (~1,000 genes or even more complex given that the accessory gene repertoire is not amenable to a straightforward

reconstruction). However, the nature of the replication and membrane machineries of LUCA remains unclear owing to the drastic differences between the respective systems of bacteria and archaea, the two primary domains of life[30–33].

The fact that the replicative DNA polymerases of bacteria, archaea and eukaryotes are not homologous has prompted ideas of an RNA-based LUCA[31,34,35]. However, the recent discovery of the structural similarity between the catalytic cores of the archaeal replicative family D DNA polymerase (PolD) and the universal DNA-directed RNA polymerase[36,37] implies a common origin of replication and transcription and suggests an 'archaeal-like' replication machinery in LUCA, with PolD serving as the replicative DNA polymerases[38]. Evolutionary reconstructions point to a fairly complex replication apparatus in the LUCA, with processive replication aided by the sliding clamp (proliferating cell nuclear antigen), clamp loader, replicative helicase and the ssDNA-binding protein. Similarly, the transcription system of the LUCA can be inferred to have already included a multisubunit RNA polymerase with the duplicated large subunits, some smaller subunits and multiple transcription regulators[39].

The membranes of archaea and bacteria consist of different types of phospholipids, namely isoprenoid ethers and fatty acid esters, respectively, with different chiralities of the glycerol-phosphate moiety[33]. Although the possibility of a membrane-less LUCA has been discussed[40,41], the general considerations on the essentiality of compartmentalization and the universal conservation of certain key membrane-associated components, such as the signal recognition particle, leave little doubt that the LUCA had membrane-bound cells[42]; however, the nature of the membrane in the LUCA remains uncertain. Phylogenomic analyses indicate that the LUCA encoded the biosynthetic pathways for both bacterial and archaeal phospholipids, implying an ancestral mixed membrane, with subsequent differentiation[30–33]. Notably, preliminary data suggest that bacteria of the Fibrobacteres–Chlorobi–Bacteroidetes group superphylum and related candidate phyla encode a complete pathway for archaeal membrane lipid biosynthesis, in addition to the bacterial fatty acid membrane pathway, suggesting that certain contemporary bacterial lineages have mixed heterochiral membranes[43]. Such a possibility is consistent with the results of recent experiments demonstrating the viability of bacteria with an engineered, mixed archaeal–bacterial membrane[44]. The same considerations apply to the cell wall, which is represented by the peptidoglycan in most bacteria[45,46] and the proteinaceous S-layer in archaea and some bacteria[47]. Importantly, however, in this case, the possibility of a wall-less LUCA cannot be dismissed.

The genetic composition of modern prokaryotes is best described in terms of the pangenome, that is, the entirety of the genes that are found in organisms with closely related core genomes that are traditionally considered to constitute a species[48–50]. The accessory genes that are present in each strain in addition to the core genome and collectively account for the bulk of the pangenome include diverse anti-parasite defence systems, genes involved in inter-microbial conflicts, such as antibiotic production and resistance, and integrated MGEs. Given that genetic parasites are intrinsic components of any replicator system, this pangenome structure should necessarily have been established at the earliest stages of cellular evolution. Thus, although important features of the LUCA remain to be clarified, we can conclude with reasonable confidence that it was a prokaryotic population with a pangenomic complexity comparable to that of the extant archaea and bacteria. The attempt on the reconstruction of the LUCA virome that we undertake here provides some insights into the pangenome of the LUCA that we discuss in the final section.

LUCA and the four viral realms

Examination of the host ranges of viruses in each of the four realms[13] and, in particular, assessment of the relationships between bacterial and archaeal members allows us to make inferences on the composition of the LUCA virome. Widespread groups with a clear dichotomy between archaeal and bacterial viruses are the best candidates for components of the virome of the LUCA. We start by mapping the major groups of viruses to the evolutionary trees of bacterial and archaeal hosts[51] (omitting eukaryotes as a derived domain of life that emerged at a later stage of evolution and is hence irrelevant as far as the LUCA is concerned[52]), thereby inferring their likely presence or absence in the LUCA virome. The results of this reconstruction (FIGS 1,2) suggest that the LUCA virome was dominated by dsDNA viruses. More specifically, several groups of tailed dsDNA viruses (*Duplodnaviria*) were assigned to the LUCA virome, indicating that (at least) this realm of viruses had already reached considerable diversity prior to the radiation of archaea and bacteria (FIG. 3). All viruses of this realm share homologous MCPs (HK97-fold), large and small terminase subunits, prohead maturation proteases and portal proteins, indicating that their morphogenetic modules are monophyletic[53–58]. Duplodnaviruses are broadly distributed among both bacteria and archaea (FIGS 1,2) and, crucially, comparative genomic analyses suggest that the archaeal and bacterial viruses within *Duplodnaviria*, on a broad scale, have coevolved with their respective hosts[59] (see discussion below).

Tailed bacteriophages are nearly universal among bacteria[60]. In archaea, duplodnaviruses or related proviruses (virus genome integrated into the cellular chromosome) have been detected in many mesophilic as well as extremophilic lineages of the phyla Euryarchaeota and Thaumarchaeota[56,61]. Furthermore, HK97-fold MCPs were identified in uncultivated archaea of the proposed phyla Aenigmarchaeota, Altiarchaeota, Nanoarchaeota, Micrarchaeota, Iainarchaeota and Asgardarchaeota (FIG. 2). However, given the potential artefacts associated with the binning of contigs from environmental genomics projects, the host assignment for these (pro)viruses should be considered with utmost caution. Nevertheless, the distribution of tailed archaeal duplodnaviruses appears to encompass highly diverse environments, mirroring the situation of their bacterial relatives and consistent with the presence of this group in the LUCA virome. Although it is difficult to precisely map specific groups of duplodnaviruses to the LUCA virome, the presence of viruses with short tails (podovirus morphology), long non-contractile tails (siphovirus morphology) and contractile tails (myovirus morphology) in both bacteria and archaea implies that all these morphologies were already represented in the LUCA virome (FIG. 3). The alternative possibility, namely that all three major groups of duplodnaviruses (that is, siphoviruses, myoviruses and podoviruses) were transferred between bacteria and archaea at later stages of evolution, cannot be formally excluded but appears less parsimonious. Furthermore, the observation that many bacterial members of the *Duplodnaviria* encode archaeal-like genome replication modules[62], which are not homologous to the bacterial functional counterparts, also argues in favour of the origin of this virus

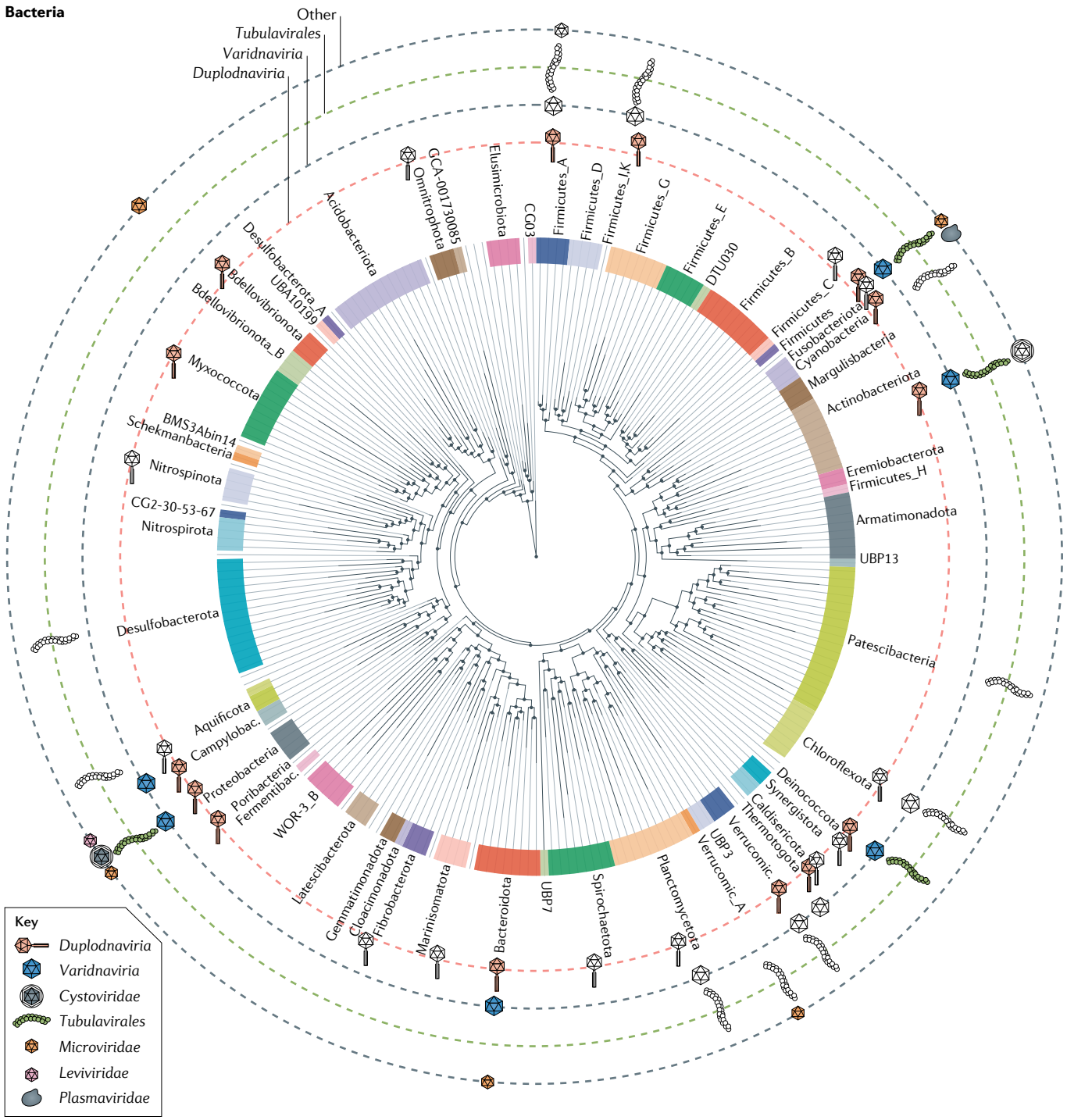
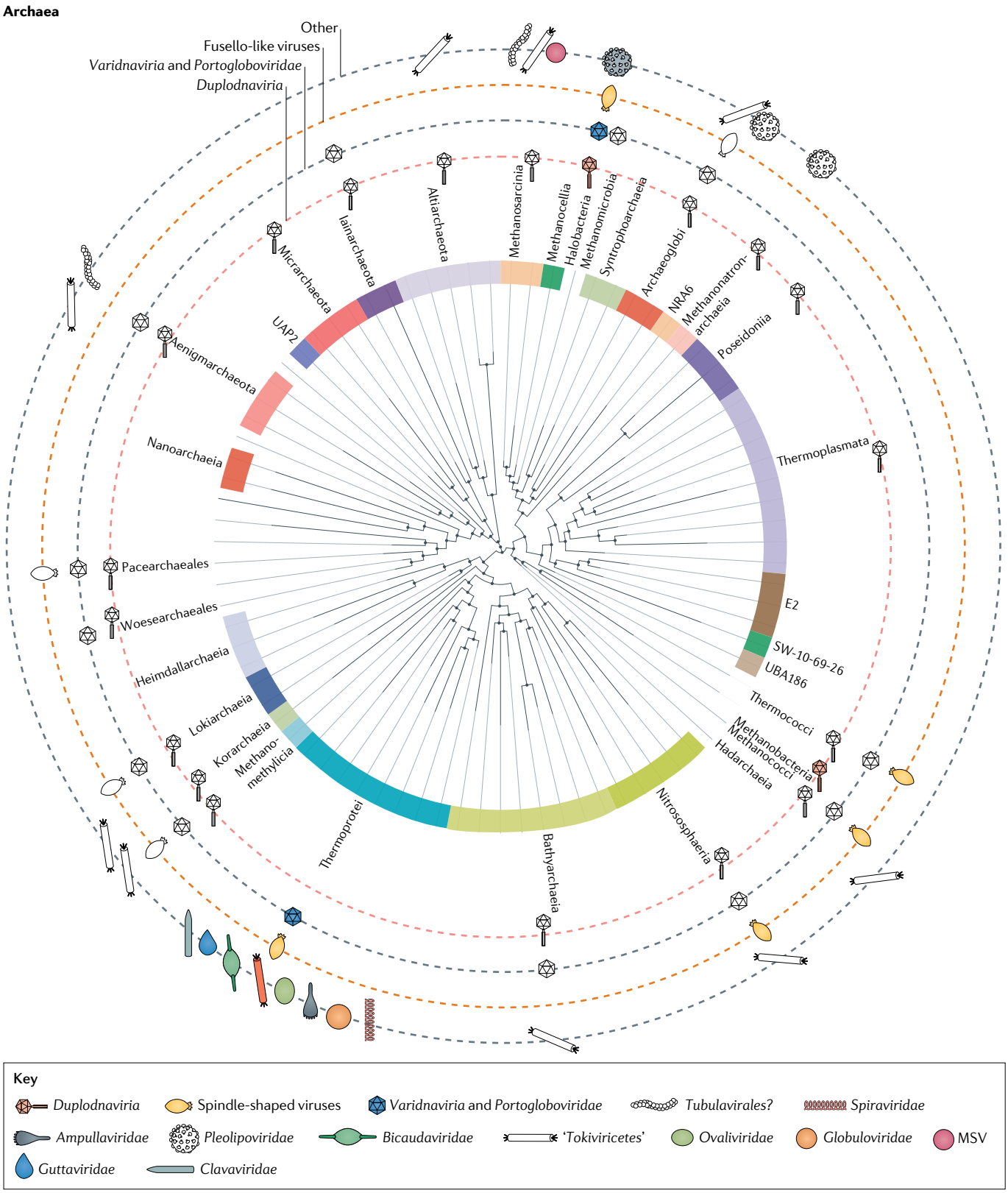


Fig. 1 | **Distribution of known viruses across the evolutionary tree of bacteria.** The figure shows the latest phylogenetic tree of bacteria, with all phyla indicated, and the major groups of viruses known to infect members of these phyla. Virus groups are represented by symbols depicting the corresponding virions. Coloured and open symbols represent virus isolates and virus genomes or putative prophages, respectively. The symbols are arranged on four concentric rings: the innermost ring depicts the distribution of members of the realm *Duplodnaviria* (families *Siphoviridae*, *Podoviridae*, *Myoviridae*, *Ackermannviridae* and *Herelleviridae*); the second ring shows members of the realm *Varidnaviria* (families *Tectiviridae*, *Corticoviridae*, *Finnlakeviridae*, *Sphaerolipoviridae* and *Autolykiviridae*); the third ring shows members of the order *Tubulavirales* (families *Inoviridae* and *Plectroviridae*); and the fourth ring includes all other virus groups, namely *Microviridae*, *Leviviridae*, *Cystoviridae* and *Plasmaviridae*. The phylogeny and taxonomic nomenclature were retrieved from the Genome Taxonomy Database[51] and visualized with AnnoTree[109]. The information on virus distribution for virus isolates with completely sequenced genomes was obtained from GenBank. The provirus distribution was retrieved from previously published work on *Duplodnaviria*[110], *Tubulavirales*[68] and *Varidnaviria*[63,64,111,112]. Supplementary Data 1 shows the known virus–host associations across the domains Bacteria and Archaea. In the spreadsheet, a genus name is indicated if a virus is known to infect (or be associated as a provirus with) any member of the phylum or class of Bacteria and Archaea, respectively.



group antedating the archaeal–bacterial divide.

The second realm of dsDNA viruses, *Varidnaviria*, is represented in prokaryotes by four families of bacterial viruses (*Tectiviridae*, *Corticoviridae*, *Autolykiviridae* and *Finnlakeviridae*), one family of archaeal viruses (*Turriviridae*) and the family *Sphaerolipoviridae*, in which different genera include viruses infecting either bacteria or archaea. However, mining metagenomic data for homologues of the DJR MCP using sensitive computational methods resulted in the discovery of a vast diversity of previously unknown viruses of this realm that, in all likelihood, infect prokaryotes[63,64]. Actual host assignments await but some of these virus genomes were found in geothermal

◀ Fig. 2 | **Distribution of known viruses across the evolutionary tree of archaea.** The figure shows the latest phylogenetic tree of archaea, with major classes or orders indicated, and the major groups of viruses known to infect members of these taxa. Virus groups are represented by symbols depicting the corresponding virions. Coloured and open symbols represent virus isolates and virus genomes or putative proviruses, respectively. The symbols are arranged on four concentric rings: the innermost ring depicts the distribution of members of the realm *Duplodnaviria*; the second ring shows members of the realm *Varidnaviria* (families *Turriviridae* and *Sphaerolipoviridae*) and family *Portogloboviridae*; the third ring shows spindle-shaped viruses (families *Fuselloviridae*, *Halspiviridae* and *Thaspiviridae* and unclassified viruses of Thermococcales); and the fourth ring consists of all other virus groups, including the candidate class '*Tokiviricetes*' (*Rudiviridae*, *Lipothrixviridae* and *Tristromaviridae*)[113] and families *Ampullaviridae*, *Ovaliviridae*, *Bicaudaviridae*, *Guttaviridae*, *Globuloviridae*, *Clavaviridae*, *Spiraviridae* and *Pleolipoviridae*, and unclassified Methanosarcina spherical virus (MSV)[114]. Putative proviruses related to bacterial members of the *Tubulavirales* were identified in some archaeal genomes[68] and are indicated with open symbols. The phylogeny and taxonomic nomenclature were retrieved from the Genome Taxonomy Database[51] and visualized with AnnoTree[109]. The information on virus distribution for virus isolates with completely sequenced genomes was obtained from GenBank. The provirus distribution was retrieved from previously published work on *Duplodnaviria*[56,66], *Varidnaviria*[63,66] and *Pleolipoviridae*[115,116]. Additional information was obtained by performing BLASTP searches[117] queried with the major capsid proteins of the corresponding viruses against the archaeal genome database at the NCBI. Supplementary Data 1 shows the known virus–host associations across the domains Bacteria and Archaea. In the spreadsheet, a genus name is indicated if a virus is known to infect (or be associated as a provirus with) any member of the phylum or class of Bacteria and Archaea, respectively.

habitats, strongly suggesting archaeal hosts[63,64]. Perhaps even more informative has been the analysis of bacterial and archaeal genomes for the presence of proviruses encoding DJR MCPs, which has substantially expanded the reach of *Varidnaviria* in both prokaryotic domains[65–67]. Phylogenetic analysis of the concatenated DJR MCP and genome packaging ATPases of archaeal varidnaviruses suggested coevolution of this group of viruses with the major archaeal lineages rather than recent horizontal transfer from bacteria[66]. Thus, most likely, the LUCA virome also included multiple groups of dsDNA viruses with vertical (both single and double) jelly-roll MCPs (FIG. 3). Furthermore, reconstruction of DJR MCP evolution sheds light on the pre-LUCA stages of virus evolution as discussed in the next section.

Among the ssDNA viruses (realm *Monodnaviria*), only members of a single order, *Tubulavirales* (until recently known as the family *Inoviridae*), consisting of filamentous or rod-shaped viruses, appear to be hosted by both bacteria and archaea. However, whereas tubulaviruses are ubiquitous in bacteria, their association with archaea was inferred from putative proviruses present in several archaeal lineages, namely methanogens and aenigmarchaea[68]. Such distribution has been judged best compatible with horizontal virus transfer from bacteria to archaea[68]. Given their ubiquity in bacteria, the origin of filamentous bacteriophages concomitantly or soon after the emergence of the last bacterial common ancestor (LBCA) appears likely, whereas their presence in LUCA cannot be ruled out either (FIG. 3). Similarly, microviruses with icosahedral capsids and circular ssDNA genomes are nearly ubiquitous in the environment and are genetically highly diverse[69–71]. Although for the vast majority of these viruses the hosts are unknown, the few known isolates infect broadly diverse bacteria from five different phyla (FIG. 1). It is thus likely that microviruses have a long-standing evolutionary history in bacteria, which probably dates back at least to the LBCA (FIG. 3).

In the extant biosphere, RNA viruses dominate the eukaryotic virome but are rare in bacteria (compared with DNA viruses) and unknown in archaea[72]. Bacterial RNA viruses are represented by two families, the positive-sense RNA *Leviviridae* and dsRNA *Cystoviridae*[60,73]. The host range of experimentally identified members of both families is limited to a narrow range of bacteria (almost exclusively Proteobacteria). However, recent metagenomics efforts have drastically expanded the known diversity of leviviruses, indicating that their share in the prokaryotic virome had been substantially under-appreciated[74,75].

Reverse-transcribing viruses are conspicuously confined to eukaryotes although prokaryotes carry a substantial diversity of non-packaging (that is, non-viral) retroelements, for example, group II introns[76,77]. The extant distribution of the viruses of the realm *Riboviria*, with its drastic display of eukaryotic over prokaryotic host ranges, might appear paradoxical given the broadly accepted RNA world concept of the origin of life[78–80], implying the early origin of RdRP and RT and, as a consequence, the primordial status of RNA viruses. The origin of leviviruses within bacteria is best compatible with their currently characterized distribution (FIG. 1) and is a distinct possibility. However, given the lack of obvious direct ancestors of the RdRP among RTs of bacterial retroelements and the ever expanding diversity of leviviruses through metagenomics[74,75], we consider that the origin of levivirus ancestors at the pre-LUCA stage of evolution and their presence in the virome of the LUCA cannot be ruled out, even if not supported by currently available data. Conceivably, at the LUCA stage and later, primordial RNA viruses were losing the evolutionary competition with the more efficient dsDNA viruses and went extinct in many lines of descent, including archaea. Under this scenario, the renaissance of the RNA viruses occurred only in eukaryotes, arguably due to the combination of barriers for DNA virus replication created by the nucleus and the emergence of the cytosolic endomembrane system that became a niche favourable to RNA virus reproduction[72].

Furthermore, unlike the LUCA, for which most evolutionary reconstructions suggest a mesophilic or a moderate thermophilic lifestyle[81,82], the last common ancestors of bacteria and archaea are inferred to have been thermophiles or hyperthermophiles[83,84]. Extremely high temperatures might be restrictive for the propagation of RNA viruses and thus could represent a bottleneck associated with the demise of the ancestral RNA virome (and potentially explain why RNA viruses are unknown in archaea)[85]. The family *Cystoviridae*, which includes dsRNA viruses, has an even narrower host range than the leviviruses, suggesting a later origin. Thus, of the realm *Riboviria*, positive-sense RNA viruses are a putative component of the LUCA virome, whereas dsRNA viruses, negative-sense RNA viruses and all reverse-transcribing viruses appear to be subsequent additions to the virus world, the latter two taxa emerging only in eukaryotes.

The ancestral status of many archaea-specific virus groups is difficult to ascertain. However, some monophyletic virus assemblages, such as those with spindle-shaped virions[16,86], infect hosts from all major archaeal lineages (FIG. 2) and thus can be traced to the last archaeal common ancestor. Therefore, their presence in the LUCA virome, with subsequent loss in the bacterial lineage, cannot be ruled out either.

Virus evolution before the LUCA

Likely cellular ancestors are identifiable for many major virion proteins on the basis of phylogenomic analyses of the corresponding protein families[87]. The reconstruction of the evolutionary paths from ancestral host

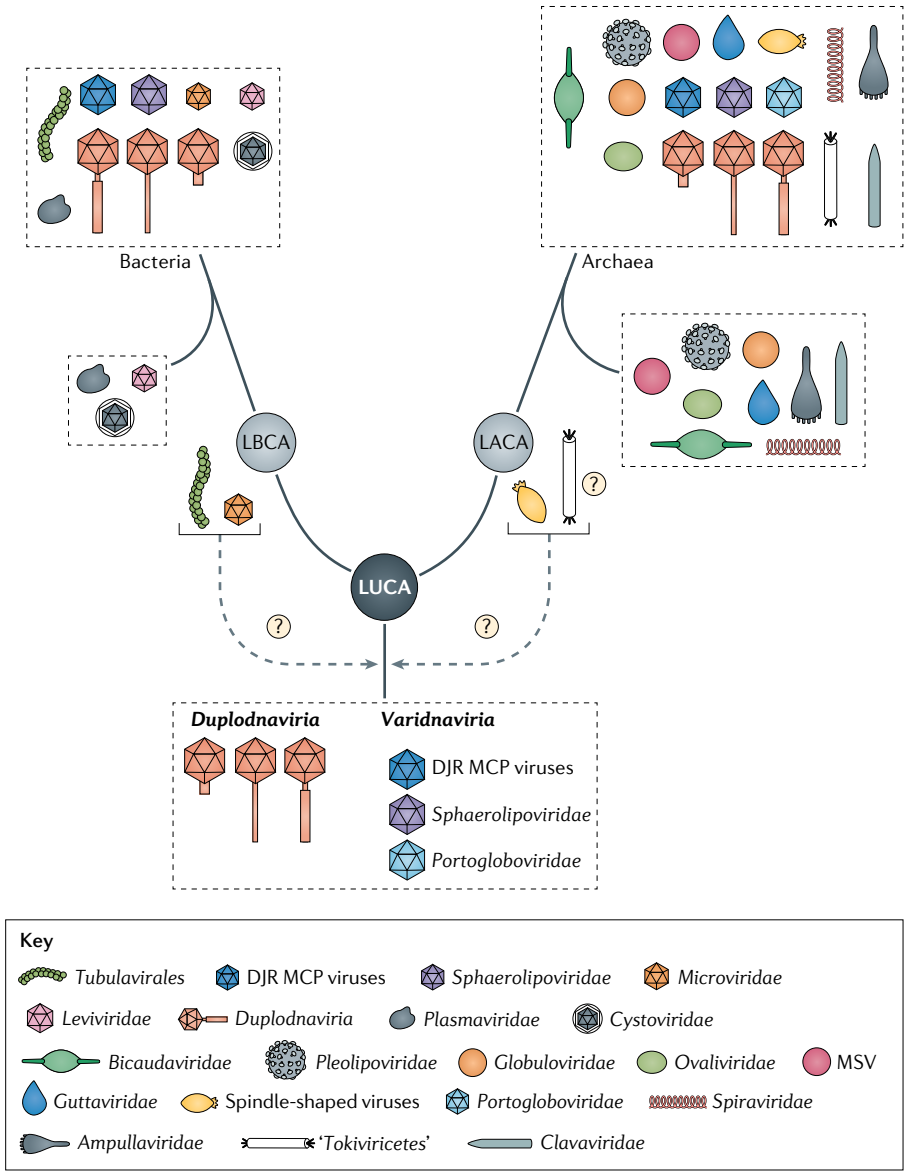


Fig. 3 | **Reconstruction of the LUCA virome from the divergence of the bacterial and archaeal viromes.** This figure shows the hypothetical complex virome of the last universal cellular ancestor (LUCA) as reconstructed from the distribution of viruses among extant phyla of bacteria and archaea. Also schematically depicted are the split of the LUCA virome into the viromes of the last bacterial common ancestor (LBCA) and the last archaeal common ancestor (LACA) as well as the subsequent diversification that resulted in the extant viromes. The divergence of bacteria and archaea from the LUCA is depicted as a bifurcation. Viruses predicted to be associated with the LBCA and the LACA are indicated next to the corresponding grey spheres. Dotted arrows indicate the possibility that the respective viruses might have been represented in the LUCA virome. DJR, double jelly-roll; MCP, major capsid proteins; MSV, Methanosarcina spherical virus.

proteins to viral capsids sheds light on the early stages of evolution of both realms of dsDNA viruses (FIG. 4). The DJR MCP of the *Varidnaviria* appears to be a unique virus feature, with no potential cellular ancestors detected. By contrast, the SJR MCP of numerous RNA viruses that was also acquired by ssDNA viruses through recombination can be traced to ancestral cellular carbohydrate-binding proteins, with several probable points of entry into the virus world[87]. Thus, the DJR MCP, in all likelihood evolved from the SJR MCP early in the evolution of viruses. Remarkably, apparent evolutionary intermediates are detectable in two virus families. Viruses in the family *Sphaerolipoviridae* encode two 'vertically' oriented SJR MCPs that are likely to represent the ancestral duplication preceding the fusion that gave rise to the DJR MCP[88–90]. The recently discovered archaeal dsDNA viruses in the family *Portogloboviridae*[91] contain one SJR MCP[92] and thus appear to represent an even earlier evolutionary intermediate (FIG. 4). Indeed, structural comparisons of the SJR MCPs from RNA and DNA viruses show that the portoglobovirus MCP is most closely related to the MCPs of sphaerolipoviruses[92]. Combined with the inferred presence in the LUCA virome of multiple groups of *Varidnaviria*, the discovery of the intermediate MCP forms in capsids of extant viruses implies extensive evolution of varidnaviruses predating the LUCA. The families *Portogloboviridae* and *Sphaerolipoviridae* appear to be relics of the pre-LUCA evolution of varidnaviruses and, accordingly, must have been part of the LUCA virome.

For the members of the second realm of dsDNA viruses, *Duplodnaviria*, no cellular ancestor was detected in the dedicated comparative analyses of the sequences and structures of virion proteins[87]. However, a recent structural comparison has shown that the main scaffold of the HK97-like MCP belongs to the strand-helix-strand-strand (SHS2) fold (with the insertion of an additional, uncharacterized domain of the DUF1884 (PF08967) family[93]) and appears to be specifically related to the dodecin family of the SHS2-fold proteins[94]. Dodecins are widespread proteins in bacteria and archaea that form dodecameric compartments involved in flavin sequestration and storage[95] and are thus plausible ancestors for the HK97-fold MCP. Although, in this case, there are no detectable evolutionary intermediates among viruses, the inferred presence of multiple groups of duplodnaviruses in the LUCA virome implies that the recruitment of dodecin and the insertion of DUF1884 are ancient events. Consistently, viruses with short tails (podovirus morphology), long non-contractile tails (siphovirus morphology) and long contractile tails (myovirus morphology) are all found in both bacteria and archaea, indicating that the morphogenetic toolkit of viruses with HK97-fold MCPs attained considerable versatility in the pre-LUCA era.

Virus replication modules

Each virus genome includes two major functional modules, one for virion formation (morphogenetic module) and one for genome replication[96]. The two modules rarely display congruent histories over long evolutionary spans and are instead exchanged horizontally between different groups of viruses through recombination, continuously producing new virus lineages.

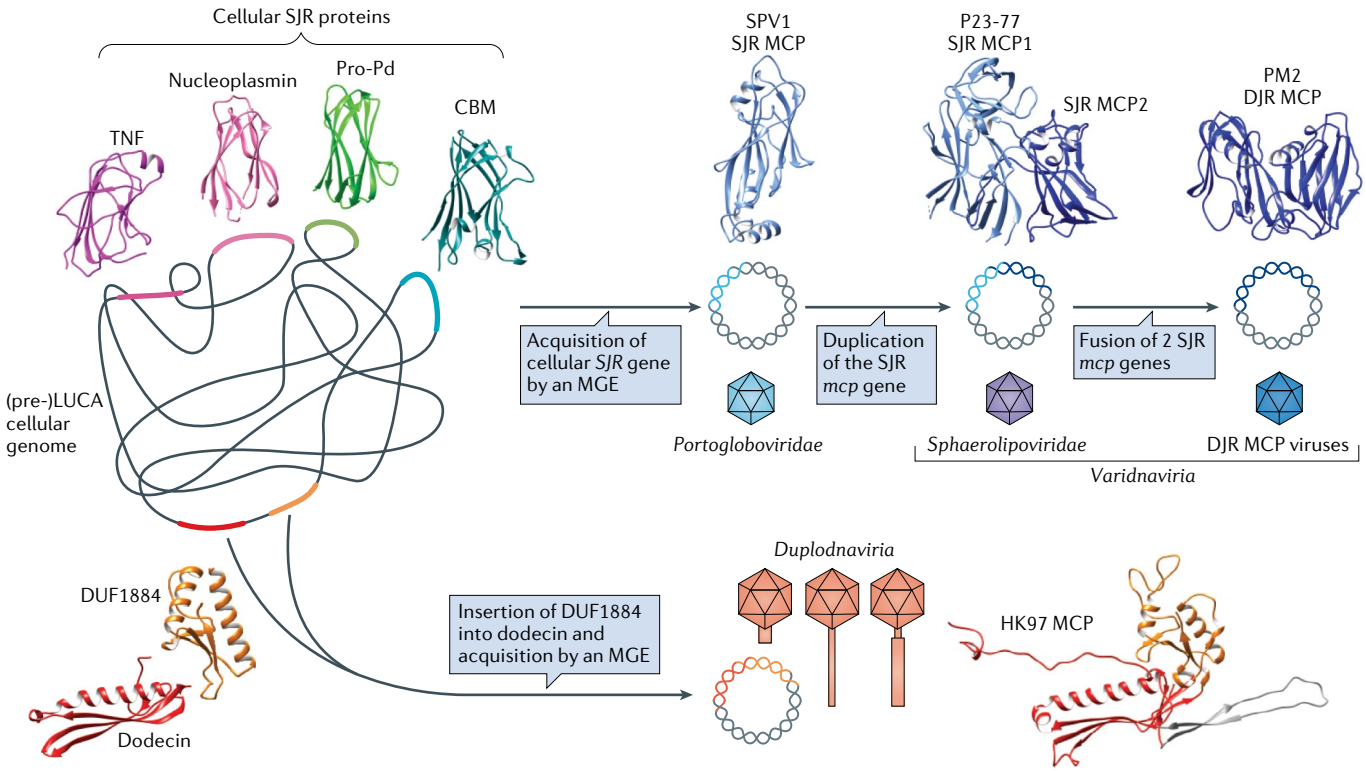


Fig. 4 | **Evolution of double-stranded DNA viruses antedating the LUCA.** The figure shows the origin of single and double jelly-roll (SJR and DJR, respectively) and HK97-fold major capsid proteins (MCPs) from cellular ancestors. The capture of the vertical SJR MCP precipitated the emergence of the virus realm *Varidnaviria*, whereas the acquisition of the HK97 MCP gave rise to the realm *Duplodnaviria*. Major evolutionary events are described next to the corresponding arrows. The likely cellular ancestors of the MCPs are shown with thick coloured lines, and the structures of the similarly coloured corresponding proteins are shown next to them: TNF superfamily protein (Protein Data Bank (PDB) ID: 2hey); nucleoplasmin (PDB ID: 1nlq); P domain of a subtilisin-like protease (Pro-Pd) (PDB ID: 3afg); carbohydrate-binding module (CBM) (PDB ID: 4d3l); dodecin family protein (PDB ID: 3qkb); and DUF1884 family protein (PDB ID: 2pk8). The SJR and DJR MCPs and the corresponding virion symbols of members of the realm *Varidnaviria* are coloured with different shades of blue. MCPs of duplodnaviruses are represented by the gp5 protein of bacteriophage HK97 (PDB ID: 1ohg); *Portogloboviridae* is represented by VP4 of Sulfolobus polyhedral virus 1 (SPV1; PDB ID: 6oj0); *Sphaerolipoviridae* is represented by a heterodimer of MCPs, VP16 and VP17, of *Thermus* bacteriophage P23-77 (PDB ID: 3zn6); and DJR MCP viruses are represented by the P2 protein of *Pseudoalteromonas* bacteriophages PM2 (family *Corticoviridae*; PDB ID: 2vvf). LUCA, last universal cellular ancestor; MGE, mobile genetic elements.

In the previous sections, we show that the morphogenetic modules including the vertical jelly-roll and HK97-fold MCPs can be traced to the LUCA virome.

One of the most widespread replication modules in the virosphere is the rolling circle replication endonuclease (RCRE) of the HUH superfamily[97]. Homologous RCREs are encoded by viruses with SJR and DJR MCPs, HK97-like MCPs and morphologically diverse ssDNA viruses and are also found in many families of bacterial and archaeal plasmids and transposons[98]. Thus, RCRE can be confidently assigned to the LUCA virome or mobilome (that is, all the MGEs of the LUCA).

Protein-primed family B DNA polymerases (pPolBs) represent another replication module with a broad distribution spanning several families of viruses and non-viral MGEs[62]. pPolB is present in bacteria-infecting members of the realms *Duplodnaviria* (phi29-like podoviruses) and *Varidnaviria* (*Tectiviridae*, *Autolykiviridae* and diverse varidnavirus genomes identified in metagenomic data) as well as in several families of archaeal viruses (*Halspiviridae*, *Thaspiviridae, Ovaliviridae* and *Pleolipoviridae*). In phylogenetic analyses, pPolBs split into two separate clades corresponding to bacterial and archaeal viruses[99,100], strongly suggesting that they have coevolved with bacterial and archaeal lineages ever since their divergence from the LUCA.

Two other key replication proteins that are among the most common in bacterial and archaeal viruses and MGEs are primases of the archaeo-eukaryotic primase (AEP) superfamily[101,102] and superfamily 3 helicases (S3H)[62]. Whereas S3H are exclusive to viruses and MGEs, the viral AEP form specific families that are not closely related to the cellular homologues. Notably, bacteria do not employ AEP for primer synthesis, and thus bacterial viruses could not have recruited this protein from their hosts. Thus, AEP and S3H, along with RCRE and pPolB, appear to represent major components of the replication modules of the LUCA virome.

More generally, contemporary duplodnaviruses display a remarkable diversity of genome replication modules, from minimalist initiators that recruit cellular DNA replisomes for viral genome replication to near-complete virus-encoded DNA replication machineries[62,103]. In many cases, these DNA replication proteins do not have close cellular homologues, suggesting a long evolutionary history within the virus world. Notably, some of the phage proteins, such as helicase loaders, have replaced their cellular counterparts at the onset of certain bacterial lineages for the replication of cellular chromosomes[104]. Although some tailed bacterial dsDNA viruses encode replication factors of apparent bacterial origin, in archaeal duplodnaviruses[57], the proteins involved in informational processes, including components of the genome replication machinery, DNA repair and RNA metabolism, are of archaeal type, with none

of the known archaeal viruses encoding components of the bacterial-type replication machinery[61,62]. Finally, tailed archaeal viruses carry archaeal or eukaryotic-like promoters[105,106], consistent with the fact that none of the known archaeal viruses encode RNA polymerases[16,107], further pointing to long-term coevolution with the hosts. These considerations argue against (recent) horizontal transfers of duplodnaviruses between bacteria and archaea accounting for the observed distribution of these viruses, even though some such transfers might have occurred. Thus, analyses of duplodnavirus and varidnavirus genome replication modules complement those of the morphogenetic modules and suggest extensive divergence of both groups of viruses in the pre-LUCA era.

Conclusions

The informal reconstructions attempted here suggest a remarkably diverse, complex LUCA virome. This ancestral virome was likely dominated by dsDNA viruses from the realms *Duplodnaviria* and *Varidnaviria*. In addition, two groups of ssDNA viruses (realm *Monodnaviria*), namely *Microviridae* and *Tubulavirales*, can be traced to the LBCA, whereas spindle-shaped viruses, most likely infected the last archaeal common ancestor. The possibility that these virus groups were present in the LUCA virome but were subsequently lost in one of the two primary domains cannot be dismissed. The point of origin of the extant bacterial positive-sense RNA viruses (realm *Riboviria*) remains uncertain, with both bacterial and primordial origins remaining viable scenarios. Further virus prospecting efforts could shed light on the history of these viruses. Although the inferred LUCA virome in all likelihood did not include members of many extant groups of viruses of prokaryotes, its apparent complexity seems to exceed the typical complexity of well-characterized viromes of bacterial or archaeal species. These observations imply that the LUCA was not a homogenous microbial population but rather a community of diverse microorganisms, with a shared gene core that was inherited by all descendant life-forms and a diversified pangenome that included various genes involved in virus–host interactions, in particular multiple defence systems.

According to the 'chimeric' scenario of virus origins, different groups of viruses evolved through recruitment of cellular proteins as virion components[19]. Here, we present evidence that — contingent on our mapping of both duplodnaviruses and varidnaviruses to the LUCA virome — several such events occurred in the earliest phase of the evolution of life, from the primordial pool of replicators to the LUCA. Moreover, virus evolution during that early era went through multiple, distinct stages as demonstrated by the reconstructed histories of the capsid proteins of the two realms of dsDNA viruses. The cellular SJR-containing carbohydrate-binding or nucleoplasmin-like proteins (the ancestors of the varidnavirus DJR MCPs) and the dodecins (the ancestors of the duplodnavirus MCPs) belong to expansive protein families that have already undergone substantial diversifying evolution prior to the origins of the two realms of viruses. The respective protein families do not belong to the universal core of cellular life, so their apparent pre-LUCA diversification further emphasizes the substantial pangenomic, organizational and functional complexity of the LUCA. This conclusion is indeed compatible with the previous inferences on the LUCA made from the analysis of coalescence in different families of ancient genes, namely that a common ancestor containing all the genes shared by the three domains of life has never existed[108].

Straightforward thinking on the LUCA virome might have envisaged it as a domain of RNA viruses descending from the primordial RNA world. However, the reconstructions suggest otherwise, indicating that the LUCA was similar to the extant prokaryotes with respect to the repertoire of viruses it hosted. These findings do not defy the RNA world scenario but mesh well with the conclusion that DNA viruses have evolved and diversified extensively already in the pre-LUCA era. The RNA viruses, after all, might have been the first to emerge but, by the time the LUCA lived, they had already been largely supplanted by the more efficient DNA virosphere.

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M.K. and E.V.K. researched data for article. M.K., V.V.D. and E.V.K. substantially contributed to discussion of content. M.K. and E.V.K. wrote the manuscript. M.K., V.V.D. and E.V.K. reviewed and edited the manuscript before submission.

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