Phages build anti-defence barriers

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Two recent studies reveal a virus anti-defence strategy whereby large, 'jumbo' phages protect themselves from both restriction endonucleases and DNA-targeting CRISPR–Cas systems, but not from RNA-targeting ones, by encapsulating the phage genome inside the cell in a 'nucleus'-like shell that is impenetrable for CRISPR effectors.

Almost all life forms, including acteria and archaea, are constantly attacked by viruses and have evolved diverse and elaborate means of defence. In response, viruses evolve counter-defence mechanisms, and so the perennial arms race goes on. Clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated proteins (Cas) are adaptive antivirus immunity systems in bacteria and archaea that function by incorporating segments of virus genomes (known as spacers) into CRISPR repeat arrays and utilizing transcripts of these spacers to recognize and then cleave the cognate virus genomes[1].

Viruses counter-act host defence strategies in different ways. The simplest strategy involves escape mutants that lose complementarity to the spacers and so evade the CRISPR response. This escape route can be efficient because the mutation rates of viruses are typically much higher than those of the hosts. However, many viruses have also evolved more specific, dedicated defence strategies; in particular, numerous anti-CRISPR proteins (Acrs) that show enormous diversity of sequences as well as structures and mechanisms of inhibition of the CRISPR systems are usually highly specific for a particular CRISPR–Cas subtype or variant[2]. Some viruses encode multiple Acrs that target different CRISPR–Cas variants, but others, albeit infecting CRISPR-carrying hosts, lack detectable Acrs altogether. Two independent studies appearing in *Nature* and *Nature Microbiology* describe a distinct, previously unsuspected anti-CRISPR strategy employed by two jumbo phages (the nickname for phages with large genomes and virions) that infect, respectively, *Pseudomonas* and *Serratia* bacteria[3,4]. Jumbo phages have been previously shown to form a proteinaceous 'nuclear' compartment that encloses the phage genome within the infected bacterial cell[5,6]. Notably, the positioning of this phage 'nucleus' (as this phage structure is unrelated to the eukaryotic nucleus, we call it simply 'shell' hereinafter) is mediated by a tubulin homologue encoded by the jumbo phages[7]. The new studies show that the shell fully protects the phages against the DNA-targeting-type CRISPR–Cas systems. The protection mechanism appears to be quite straightforward: the phage shell is physically impenetrable for the CRISPR effector complexes, as shown by direct observation of the localization of these complexes in the bacterial cytosol and their exclusion from the jumbo phage shells. In accordance with this physical shielding mechanism, the jumbo phages possess 'pan-CRISPR' resistance; that is, they protect the phage genome against all tested DNA-targeting CRISPR–Cas systems, including those that are not encoded by the host. Moreover, the phage shell also excludes restriction endonucleases, suggesting that it functions as a general anti-defence barrier (Fig. 1a). This notion is further supported by the observation that *Pseudomonas* bacteria acquire no spacers from jumbo phages, whereas numerous spacers against other phages accrue[3].

Notably, the barrier is selective. When a restriction endonuclease is fused to a phage DNA-binding protein, it is internalized within the shell and cleaves the phage genome; however, the same does not work for CRISPR–Cas because the effector complexes are too large. Nevertheless, these findings indicate that the virus shell is a molecular machine capable of importing proteins that function inside while blocking host defences[3].

By contrast, reproduction of jumbo phages is efficiently inhibited by the type III systems that target both RNA and DNA, and type VI CRISPR–Cas systems[3,4]. Notably, this is the first direct validation of the original hypothesis that type VI systems target RNA transcripts of double-stranded DNA viruses and abrogate their reproduction. A search of bacterial genomes for spacers matching phage genomes revealed many spacers targeting jumbo phages in type III but not type I CRISPR arrays4. Type III systems are thought to capture jumbo phage-targeting spacers via the activity of the reverse transcriptase that is fused to the Cas1 protein in many type III systems and catalyses spacer acquisition from virus RNA[8].

The demonstration of the protective function of the shells in two jumbo phages reveals a new type of virus-encoded antidefence mechanism and sheds light on the evolution of RNA-targeting CRISPR–Cas systems. One of the key forces behind the emergence of RNA targeting could be avoidance of the physical barriers protecting virus genomes. How common are such barriers among viruses? Further investigation is required to address this question, but the recent discovery of 'megaphages' encoding tubulin homologues[9] suggests that they form protective shells as well, and that many such viruses could be lurking in the dark matter of the Earth's virome.

From a more general perspective, building a protective shell around the genome is certainly not a prerogative of large phages. Structures with similar functions seem to have evolved on multiple, independent occasions. Thus, in double-stranded RNA viruses, genome replication and transcription occurs within capsids without the genome ever being exposed to the host cytosol, therefore protecting it from host defences such as RNA interference. Similarly, genome replication of many positive-sense RNA viruses takes place within virus-induced spherules, which are small membrane invaginations that protect the double-stranded replication intermediates[10]. Conversely, cellular organisms protect themselves from viruses under the same principle. The eukaryotic nucleus is a highly complex organelle with multiple functions but may have largely evolved as a selective barrier to prevent the access of DNA viruses to the host replication–transcription machinery (Fig. 1b). Some viruses, such as parvoviruses, polyomaviruses and herpesviruses, have evolved means to cross the barrier, whereas the diverse large DNA viruses, such as poxviruses or mimiviruses, replicate in the cytoplasm within complex structures known as 'virus factories' that are sometimes described as 'nuclear-like' organelles[11]. It remains to be investigated whether virus factories in eukaryotes provide protection against host defences, as in the case of jumbo phages. Nevertheless, the evolution of the nuclear compartment appears to have substantially affected the composition of the eukaryotic virome that is dominated by RNA viruses[12] (Fig. 1b). Thus, although the protein components of the eukaryotic nucleus and the 'nucleus'-like shell of large phages are unrelated, there seems to be a deep functional analogy between these means of cellular defence and viral anti-defence.

Competing interests

The authors declare they have no competing interests

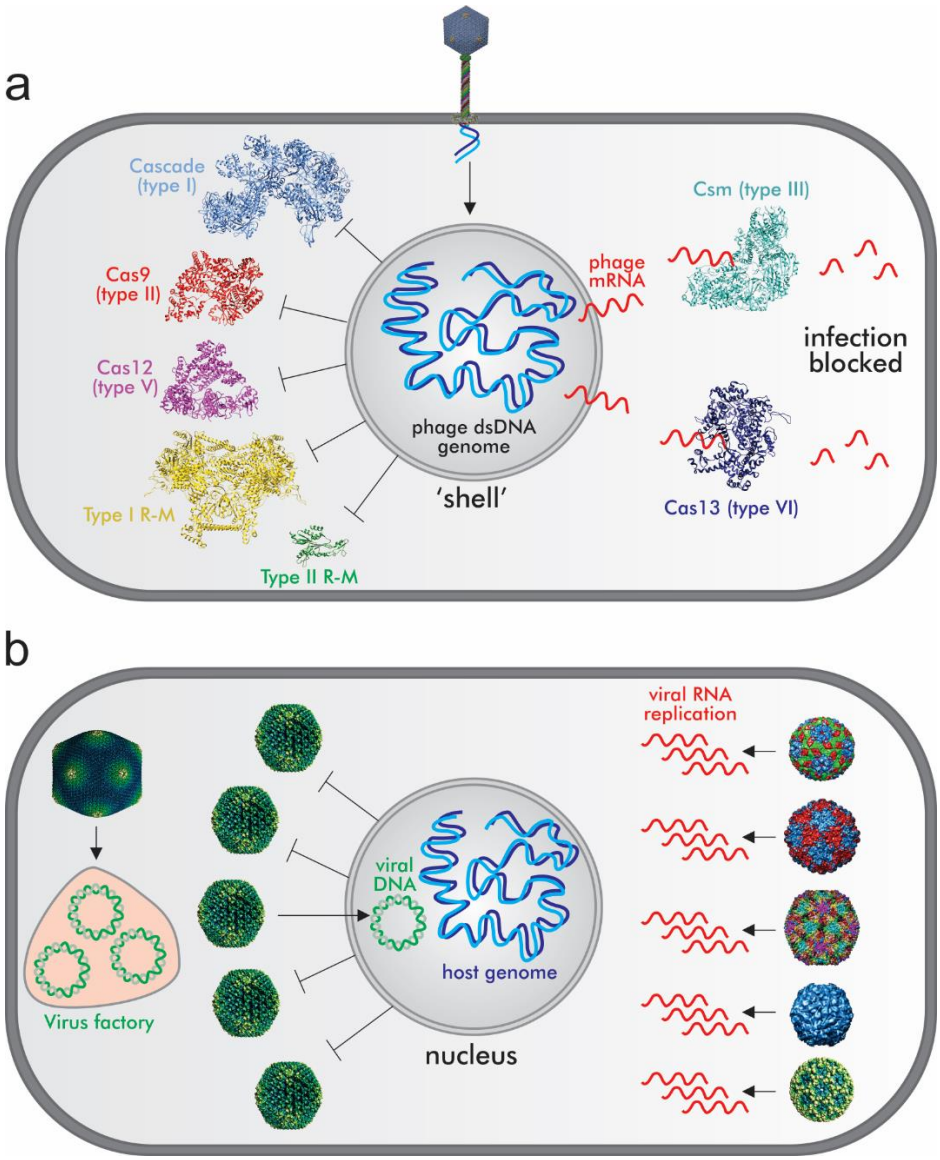

Figure 1. 'Nucleus'-like organelles in host defence and virus anti-defence. **a**, Proteinaceous shells protect genomes of large phages from host defences active against double-stranded DNA targets. By contrast, CRISPR–Cas systems of types III and VI, which cleave RNA, are not affected by the shells and efficiently restrict virus propagation. R–M, restriction–modification system. dsDNA, double-stranded DNA **b**, The eukaryotic nucleus protects the host from most DNA viruses. Only those DNA viruses that have evolved the means to either penetrate the nuclear envelope or autonomously replicate their genomes in the cytoplasm (within virus factories) have persisted in eukaryotic hosts. Conversely, cytoplasmic replication, typical of most RNA viruses, likely promoted their evolutionary success and diversification in eukaryotes. The structures shown in **a** are reproduced from the Protein Data Bank (PDB): Cascade PDB ID: 4u7u; Cas9 PDB ID: 4cmp; Cas12 PDB ID: 5ng6; Type II R-M PDB ID: 1eri; Csm PDB ID: 6mur; Cas13 PDB ID: 5xwy. Type I R-M is adapted from model coordinates detailed in ref. 13; virion images in **b** reproduced from VIPERdb.

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