COMMENTARY

Virophages go nuclear in the marine alga *Bigelowiella natans*

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The virus world in which we live will never cease to amaze us. Historically, viruses were studied primarily from medical and economic perspectives; however, the last decades have shown that viruses play roles far more versatile than we could have imagined. Being the most abundant biological entities on the planet (there are ~10^{10} virus particles in every liter of seawater), viruses drive biogeochemical cycles on a global scale (1), they affect biodiversity and community structures of their hosts [for which they can even act as symbionts (2)], and perhaps most profoundly, viruses have influenced all cellular life from its very beginning and they continue to leave their footprints in cellular genomes (3, 4), including our own. One of biggest surprises in recent virological history was the discovery of *Acanthamoeba polyphaga mimivirus* and other giant viruses, whose particles can be seen under a light microscope and contain DNA genomes that code for more than 1,000 proteins (5). As it turns out, giant viruses are rather common in the environment, infecting freshwater amoebae as well as marine heterotrophic and photosynthetic protists (6). Another unexpected finding was that giant viruses in the family *Mimiviridae* were associated with a previously unknown group of smaller double-stranded DNA (dsDNA) viruses that acted as parasites of the former. Dubbed "virophages," these icosahedrally shaped viruses with 20- to 30-kilobase-pair (kbp) genomes replicate in the cytoplasmic virus factories of their giant viruses, where they exploit the transcriptional machinery of their viral host (7). To replicate, virophages must therefore infect a susceptible host cell that is coinfected with a permissive giant virus, i.e., a virus that has the capacity to support gene expression of the virophage. Virophages appear to have evolved multiple strategies to track down their viral and cellular hosts. The Sputnik virophage can adhere to long fibers on the surface of the mimivirus capsid, and it is assumed that Sputnik hitches a ride when mimivirus is phagocytosed by the amoebal host cell (8). By contrast, the mavirus virophage enters the host cell independently of its giant virus CroV, which lacks an external fiber coat (9). Another mechanism by which a virophage can stay in touch with its host cell or giant virus is to insert its genome into either host genome. Whereas Sputnik integration into the mimivirus genome has been described (10), no provirophages (i.e., integrated virophage genomes) in a eukaryotic genome have been found so far. In PNAS, Blanc et al. (11) now report such a case.

The authors searched through more than 1,000 eukaryotic genomes for signatures of virophages, and they struck gold in the unicellular alga *Bigelowiella natans*. *B. natans* belongs to a group of mixotrophic protists called chlorarachniophytes that have trodden an interesting evolutionary path. Their

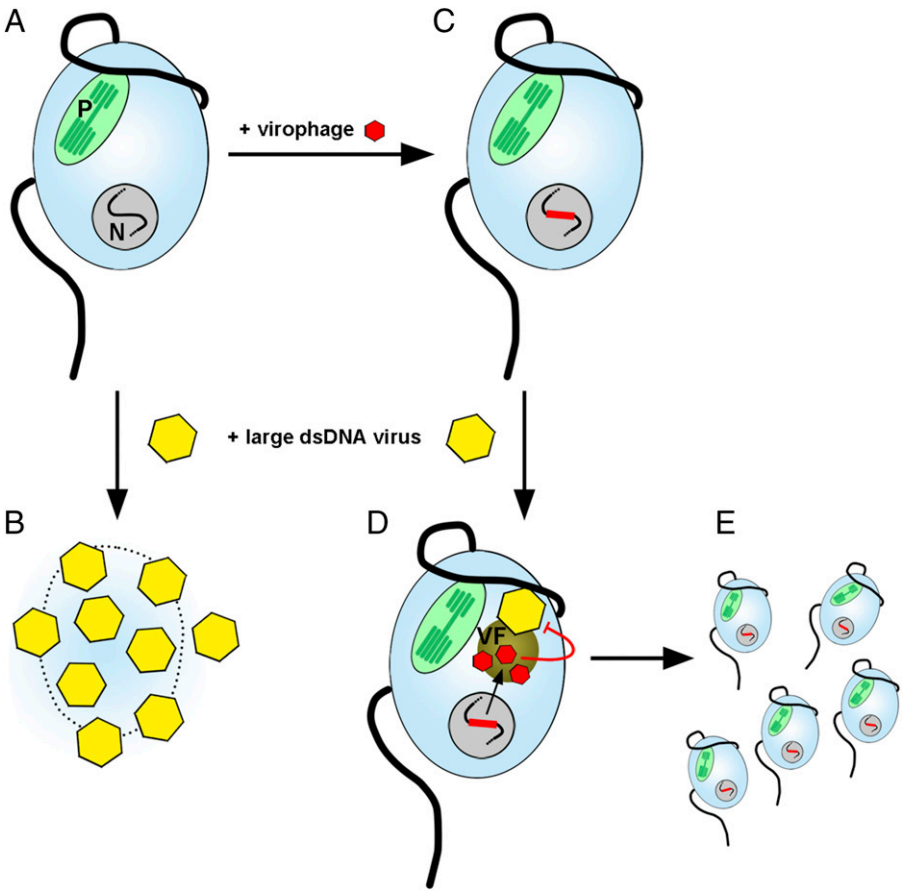

Fig. 1. A model for the protective effect of provirophages on their host cells. Modified from ref. 11. Shown is a schematic *B. natans* cell (*A*), which can be infected and lysed by a large dsDNA virus (*B*). Blanc et al. (11) propose that a virophage could integrate into the nuclear genome of a host cell in the absence of a large dsDNA virus. The provirophage-carrying cell (*C*) does not produce virophage particles until the cell is infected by a large dsDNA virus, which is then inhibited in its infection cycle by the reactivated virophage (*D*). As a result, the host population may survive the viral infection and disseminate the provirophage (*E*). N, nucleus; P, plastid; VF, virus factory.

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plastids are derived from secondary endosymbiosis, i.e., their heterotrophic ancestor engulfed a eukaryotic cell (a green alga in this case) that already possessed a chloroplast of cyanobacterial origin (12). Remarkably, not only did chlorarachniophytes retain the envelopes of those enslaved organisms (their plastid is surrounded by four membranes), but they still contain remnants of the green algal endosymbiont's nucleus, which is referred to as a nucleomorph. *B. natans* therefore harbors four distinct genomes that are located in the nucleus, the mitochondrion, the plastid, and the nucleomorph. Following the uptake of the green algal endosymbiont, substantial gene transfer to the host nucleus occurred, which left the plastid and nucleomorph genomes with mere 57 and 284 protein-coding genes, respectively. Amid the resulting mosaic nuclear genome of *B. natans* (13), Blanc et al. identified 38 virophage-like elements. Although these elements ranged from extremely truncated gene snippets of just 100 bp to presumably complete virus genomes of more than 30 kbp, they were highly similar at the nucleotide level, indicating that they derived from a common ancestral virophage. It is difficult to estimate how much time has passed since the *B. natans* genome was invaded by these viral elements, but the fact that the viral sequences display a lower G+C content than the flanking host sequences implies that the integration events happened recently enough to prevent complete assimilation to the host nucleotide composition.

Blanc et al. then analyzed gene expression data of several *B. natans* strains and found most of the ∼300 virophage genes to be transcribed—a surprising finding because virophages are thought to rely on the transcription enzymes of a coinfecting giant virus. Whether this transcriptional activity is indicative of a biological mechanism that might benefit virophage or host cell remains to be tested. However, based on the protective effect that virophages like Sputnik or mavirus have on their host cell populations in the presence of giant viruses (7, 9), it is tempting to speculate that the *Bigelowiella* provirophages could interfere with the replication of a *B. natans*-infecting large DNA virus. Blanc et al. propose a scenario in which virophages could enter the host cell independently of a giant virus and integrate into the host genome. Tied to chromosomal DNA replication of the cell, the virophage would be removed from the constant race against decay by adverse environmental conditions such as UV light, before it encounters a new giant virus-infected host. Upon superinfection with a permissive giant virus, the provirophage would become active again and interfere with the production of new giant virus particles (Fig. 1). Indeed, this scenario would describe a mutualistic relationship between a virus and a host cell (2): the host cell provides an opportunity to the virophage to persist and spread vertically within the host population, and, in return, the virophage protects the host cell from lysis by giant viruses (9, 11).

The fact that no giant viruses infecting *Bigelowiella* have been described to date does not weaken this hypothesis. Remarkably, not only does the *B. natans* genome harbor virophage-like elements, it also contains pieces of putative large DNA virus genomes (11). The largest such fragment is 165 kbp long and contains 83 genes, most of which are unknown, but some display clear phylogenetic affinities to large algal viruses. In contrast to the virophage-like elements, the large virus insert was found to be transcriptionally silent and had a G+C content similar to that of the host nuclear genome (11). This algal lineage has apparently witnessed multiple encounters with DNA viruses of various sizes. Blanc et al. demonstrate how paleovirology can reveal stories of long ago battles between viruses and their hosts, even between different viruses.

There is yet another, more far-reaching twist to the story. Some virophages have strong genetic ties with 15- to 25-kbp-long insertion elements, which have been named Maverick or Polinton DNA transposons (9, 14–16). These endogenous elements are widely distributed in the eukaryotic domain and stand out from most other DNA transposons due to a set of conserved genes with undeniable virus-like properties. Two of these genes were recently found to be distant versions of the jelly-roll capsid proteins found in many DNA viruses such as pox- and adenoviruses, as well as virophages (17). The Maverick/Polinton transposons can therefore be viewed as endogenous viruses, and these "polintoviruses" may even have played a pivotal role in the evolution of several families of eukaryotic DNA viruses, including giant viruses and their virophages (18). The *Bigelowiella* provirophages could represent an intermediate form between freely replicating virophages and the endogenous Maverick/Polinton elements and may shed more light on the flow of mobile genetic elements in the microbial world.

Overall, the findings by Blanc et al. raise several interesting questions, such as how frequently and under which conditions a virophage can stably integrate into a host genome. Unclear is also whether genome integration is a dead end for the virophage, or whether the provirophage can be triggered to resume active replication by an incoming giant virus. The most interesting question in this context may be whether provirophages play a role in defending protist populations from giant virus infections. The discovery of provirophages in the *B. natans* genome strongly suggests the existence of chlorarachniophyte-specific giant viruses. Isolation of such a virus in laboratory culture may provide answers to at least some of these riddles.

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