New and Notable

Physical Evolution of Pressure-Driven Viral Infection

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Viruses are among the simplest biological objects. They typically consist of a container, called a capsid, which holds the viral genome. Despite their simplicity, viruses exhibit extreme diversity and are highly prone to mutations, which radically limit the efficiency of contemporary antiviral therapies. Many antiviral therapies are focused on preventing a virus from entering or leaving its host cell. Other strategies focus on interfering with the action of virally encoded enzymes necessary for genome replication and protein expression. Because of their high biomolecular specificity, many of these therapies fail when the virus mutates.

Physical virology presents a new approach that aims to provide a physical description of common mechanisms controlling viral replication for a broad range of viruses. The idea to search for physical generalities in viruses with regards to genome release and encapsidation is rather new. The viral genome of motor-packaged double-stranded (ds) DNA viruses is a microns-long, charged molecule enclosed in a rigid protein capsid a few tens of nanometers in diameter. This applies to the majority of bacterial viruses (bacteriophages), as well as some eukaryotic viruses (e.g., herpesviruses). At high packaging densities, encapsidated DNA is strongly bent and DNA-DNA interactions are mainly

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mediated by hydration forces (the force required to remove water molecules from DNA to the bulk solution), which dominate the electrostatic interactions [\(1](#page-1-0)). This physical situation leads to high osmotic pressure within the capsid.

Exactly one decade ago, it was theoretically proposed that because all viruses are permeable to water and ions, a complete suppression of the viral genome ejection can be achieved by creating an osmotic pressure gradient between the host solution and the DNA within the capsid [\(2](#page-1-0)). This hypothetical experiment was confirmed for the first time when DNA ejection was osmotically suppressed in bacteriophage λ with an osmotic stress polymer, polyethylene glycol (PEG) ([3](#page-1-0)). By determining the PEG concentration required to completely suppress genome ejection, an internal capsid pressure of tens of atmospheres was verified ([3\)](#page-1-0). Similar osmotic suppression measurements were later performed on phages T5 [\(4](#page-1-0)) and SPP1 [\(5](#page-1-0)), confirming capsid pressures of tens of atmospheres in all of these bacterial viruses. These experimental observations have raised a conceptual question: how universal is the pressure-driven DNA ejection mechanism for viruses with doublestranded genomes?

In an article published in this issue, Hanhijärvi et al. [\(6](#page-1-0)) are addressing this fundamentally important question in virology by designing a single-molecule assay for osmotic suppression of dsDNA ejection from an archaeal virus His1. Archaeal viruses present an important system from the viral evolution standpoint because their archaeal host cells share common characteristics with both bacteria and eukaryotes [\(7](#page-1-0)). The osmotic suppression assay described above presents a universal approach to map internal pressures in viruses. It has so far been limited to phages for which membrane receptors could be purified and solubilized in solution without losing their ability to trigger DNA ejection from phage in vitro. The authors discovered a way to trigger DNA ejection from His1 virus in vitro by using a nonspecific, mild detergent treatment with Triton X-100. They also demonstrate with restriction enzymes that DNA ejection has directionality, where the left end of the genome is ejected first. This suggests that DNA ejection occurs without degradation of the major capsid proteins.

The authors quantify the extent of DNA ejection by attaching His1 particles to the glass coverslip in a microfluidic chamber. The ejected DNA is stretched under the buffer flow and visualized using SYBR gold dye. The addition of PEG suppresses DNA release and inhibits ejection completely at ~10 atm. Furthermore, they show that the addition of Mg^{2+} -ions also inhibits the extent of genome ejection by permeating the capsid and screening the repulsive interactions between the encapsidated DNA strands, also found with phage λ [\(8](#page-1-0)).

The article also discusses the fact that pressure-driven DNA ejection from His1 is incomplete in vitro even in the absence of an external osmotic pressure. This suggests that additional forces are required for complete internalization of the genome in vivo. Such additional pulling forces may be arising from DNA binding proteins present in the cellular cytoplasm. However, one should also note that in the in vitro single-molecule experiments described in this article, the DNA ejected from His1 is stretched by the buffer flow in the microfluidic chamber. At the same time, in vivo, it was experimentally verified that molecular crowding of the cellular cytoplasm always condenses the DNA due to an excluded volume effect, which is directly related to the osmotic pressure [\(9,10\)](#page-1-0). It was previously shown for phage λ , that the host solution's osmotic pressure causes ejected DNA to condense, resulting in a force that

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pulls DNA out of the capsid (11). The physical origin of this pulling force is minimization of the surface energy of the DNA condensate. It would therefore be important to verify in the future whether this is also the case for His1. The ejected His1 DNA could be allowed to condense in the presence of crowding agents mimicking archaeal cytoplasm.

The packaging volume fraction of dsDNA in His1 of $~50\%$ is similar to that in tailed dsDNA bacteriophages. This high packaging density leads to nonspecific DNA-DNA repulsive interactions and genome bending stress resulting in high capsid pressures. The discovery of pressure-dependent DNA ejection in viruses infecting cells from different domains of life (archaea and bacteria) demonstrates that it is an evolutionarily conserved physical mechanism, which is a key to viral replication. Furthermore, this physically stressed state of the viral genome is unique and is not present elsewhere in the cell. Therefore, it provides a new target for antiviral drug development that is resistant to viral genome sequence mutations.

Various aspects of genetic evolution have been the focus of investigation in the field of virology. A comparative examination of genomes of dsDNAtailed phages indicates that by horizontal exchange of genetic sequences, phages are genetically related to each other and probably to viruses of eukaryotes and archaea (12). The physical aspects of the evolution of viruses are significantly less understood. Recently, Belshaw et al. (13) have verified that a physical limit on genome length by the viral capsid has led to gene overlap evolving as a mechanism for producing more proteins from the same genome length. This demonstrates how a genetic mechanism has evolved from a physical constraint of the packaged genome density. Similarly the genome packaging density value is unique for a virus, because it not only determines the number of genes but also creates the internal pressure required for genome delivery.

Besides the pressure-dependent genome delivery, the infectivity of the virus depends on its ability to survive the stress of the external environment between infections. The internal osmotic pressure resulting from tight DNA packaging provides an enhanced stability to the viral capsid against rupture in the extra- and intracellular environment (14). Viruses transmitted through the environment have to survive outside of their hosts. Specifically, they have to adapt for extended periods of time in harsh conditions before meeting a susceptible cell. Various physical factors strongly affect viral transmission and infectivity, such as temperature, UV radiation, pH and salt, and osmotic pressure, among others (14). Archaeal viruses have evolved to withstand extreme environments because their host cells are often extreme thermophiles or extreme halophiles (living in very high concentrations of salt), such as His1 virus (7). Intra- and extracellular environments affect viral development at both genetic and physical levels. Correlating genome confinement and mechanical capsid stability with the efficiency of viral replication in archaeal viruses provides a unique approach to verify the connections between physical and genetic evolution. The research by Hanhijärvi et al. (6) is a pioneering step in this novel direction.

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