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# Bacteriophage P22 ejects all of its internal proteins before its genome

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ABSTRACT

Double-stranded DNA bacteriophages are highly pressurized, providing a force driving ejection of a significant fraction of the genome from its capsid. In P22-like *Podoviridae*, internal proteins ("E proteins") are packaged into the capsid along with the genome, and without them the virus is not infectious. However, little is known about how and when these proteins come out of the virus. We employed an *in vitro* osmotic suppression system with high-molecular-weight polyethylene glycol to study P22 E protein release. While slow ejection of the DNA can be triggered by lipopolysaccharide (LPS), the rate is significantly enhanced by the membrane protein OmpA from *Salmonella*. In contrast, E proteins are not ejected unless both OmpA and LPS are present and their ejection when OmpA is present is largely complete before any genome is ejected, suggesting that E proteins play a key role in the early stage of transferring P22 DNA into the host.



## Introduction

The detailed mechanisms by which bacteriophages deliver their genomes into their bacterial hosts are not fully understood. In general, for tailed double-stranded (ds) DNA phages, proteins located in the phage tail first contact the surface of the bacterium and the phage diffuses along the surface until it finds a specific receptor. Upon binding to the receptor, the phage tail undergoes a series of conformational changes that result in release of the DNA and in its translocation from the capsid into the cytoplasm of the host (Poranen et al., 2002; Bhardwaj et al., 2014). In order to overcome the defense barriers of the bacteria – *e.g.*, the outer and inner membranes along with the periplasmic space in between – bacteriophages use different strategies based on their tail morphology. Compared to the long-tailed *Myoviridae* (with contractile tails) and *Siphoviridae* (non-contractile tails) (Leiman and Shneider, 2012; Davidson et al., 2012), *Podoviridae*, a family of bacteriophages with tails shorter than the width of the periplasm, cannot directly use their tails to penetrate both membranes. While studies of the particular podoviruses phi29 and T7 (González-Huici et al., 2006; Molineux, 2001) have shed much light on their mechanism of infection, to date no generalized mechanism of infection for phages has been identified.

P22 is a member of *Podoviridae* that infects *Salmonella enterica*. It is a dsDNA phage with a 43.5 kbp genome that is packaged via a headful mechanism (Casjens and Hayden, 1988) into an icosahedral procapsid formed from assembly of the coat, scaffolding, and portal proteins (King et al., 1976). There are three internal proteins (called "pilot", "ejection", or "E" proteins) packaged inside P22 procapsids, all incorporated by the scaffolding protein in the early stages of assembly: gp16, gp20 and gp7 ("gp"=gene product), each with 10–20 copies (Israel, 1977). A short tail "machine" is then connected at a unique five-fold vertex to complete the P22 virion. Several of these structural proteins have been identified in recent cryoEM investigations (Tang et al., 2011; Lander et al., 2009). Although density in these reconstructions had been ascribed to the E proteins (Chang et al., 2006; Lander et al., 2006), a crystal structure of the complete portal, as well as the most recent asymmetric reconstructions (Olia et al., 2011; Tang et al., 2011) show this to be incorrect. Accordingly, the location of the three E proteins within the mature virion remains unknown.

Along with their DNA, most phages eject proteins that have been packaged into the capsid, and this is generally essential for

*Abbreviations:* LPS, lipopolysaccharide; PFU, plaque forming units; PEG, polyethylene glycol; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; OmpA, outer membrane protein A

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infection (Molineux and Panja, 2013). Although the exact roles that each of these E proteins plays in infection by P22 have not been determined, some information is available. For example, cells infected with gp16-deficient phage (a mutant lacking a functional gp16 E protein) continue to divide normally and do not replicate the P22 genome, but co-infections show that gp16 can work *in trans* to complement a gp16-deficient particle, indicating an early function (Hoffman and Levine, 1975a, 1975b). Furthermore, gp16-deficient phages do not induce the superinfection-exclusion response and gp16 is not part of the replication machinery (Israel et al., 1972). Gp7 and gp20 often co-purify with gp16, and gp7 ejection does not occur when gp16 is absent (Israel, 1977). Indirect evidence supports a membrane-breaching role because purified gp16 disrupts dye-loaded lipid vesicles (Perez et al., 2009). It has been proposed that the E proteins protect the DNA in the periplasm during infection (Israel, 1977), but their function and location post-infection have yet to be determined experimentally. Taken together, these facts suggest that the E proteins, gp7, gp16, and gp20, may be linked to the efficiency and dynamics of DNA ejection across host membranes.

If the receptor is known and can be solubilized, phage ejection can be triggered *in vitro*, in which case the extent of DNA ejection can be controlled by the presence of an osmolyte in the surrounding buffer solution (Tzlil et al., 2003; Evilevitch et al., 2003; Castelnovo and Evilevitch, 2007; Bauer et al., 2013). More explicitly, each concentration of high-molecular-weight polyethylene glycol (PEG8000) in the host ("external") solution corresponds to a certain amount of water being drawn out of the phage capsid (from which PEG is excluded). As a result, the water inside is under tension, thereby producing a force resisting the ejection of DNA. Such osmotic suppression studies done with dsDNA phages like lambda (Evilevitch et al., 2003) and T5 (Leforestier et al., 2008), both members of *Siphoviridae*, have shown that the virus capsid is highly pressurized as a result of the electrostatic self-repulsion and bending of the densely-packed, negatively-charged, semi-rigid DNA (Riemer and Bloomfield, 1978; Tzlil et al., 2003). In these *in vitro* experiments, in which pressure is the only driving force for DNA ejection, the length of DNA ejected can be tuned by the osmotic pressure difference between the outside and inside of the capsid (Evilevitch et al., 2003). In the present study we use osmotic suppression to examine for the first time the ejection of both the DNA and the E proteins from P22, which sheds light on ejection mechanisms in *Podoviridae*.

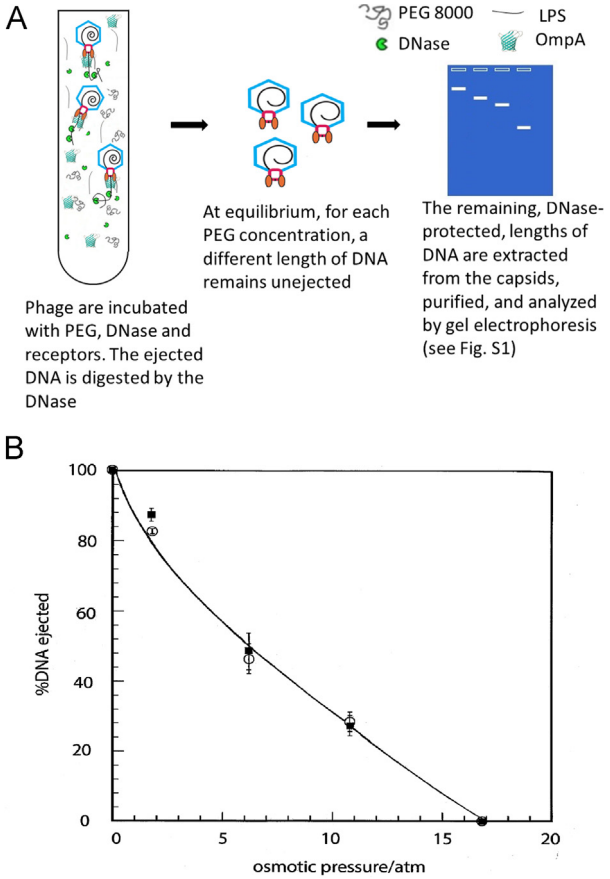


**Fig. 1.** The extent of genome ejection is controlled by osmotic pressure. (A) Cartoon of P22 *in vitro* ejection. The purified phage are incubated in PEG8000 and DNase. Receptor is then added, triggering ejection of progressively less DNA as the osmotic pressure increases; ejected DNA is digested by the DNase, and the protected DNA that remains in the capsid is extracted and its length analyzed by gel electrophoresis. (B) Measured DNA ejection percentage from P22 at various osmotic pressures under different receptor conditions: P22+LPS (○); P22+LPS+OmpA (■). Ejection is triggered by addition of receptor (LPS, or LPS and OmpA) in the presence of PEG and DNase I; the DNase is inactivated; and the DNA remaining in the capsids is extracted, run on an agarose gel, and the unejected length calculated from an accompanying DNA ladder. % DNA ejection is relative to the full-length DNA. The solid curve is drawn to aid the eye.

## Results and discussion

For P22, the O-antigen portion of lipopolysaccharide (LPS) located on the surface of the host *S. enterica* serovar Typhimurium works as a primary receptor for infection (Iwashita and Kanegasaki, 1973). Further, it has been demonstrated (Andres et al., 2010) that LPS can trigger a slow ejection of DNA from P22 *in vitro*, which makes it possible to study the process with the osmotic suppression technique.

### *Osmotic suppression experiments show release of DNA from P22 is inhibited at a pressure of 16.8 atm*

Using LPS to trigger ejection, we determined the fraction of the DNA remaining unejected in the presence of an osmotic pressure by separating the capsids from the ejected DNA, which was degraded by DNase, and then recovering and analyzing the DNA remaining in the capsids as in the experiment of Evilevitch et al. (2005). Fig. 1A is a cartoon showing the ejection behavior of P22 in the presence of LPS under different osmotic pressures. Ejected DNA is digested into nucleotides, but the DNA that is kept inside the capsids by the PEG pressure is protected; this DNA is extracted (see *Materials and methods*) and its length analyzed by gel electrophoresis. As a control, we have demonstrated that the isolated P22 genome can be degraded by DNAse in the presence of PEG8000, as is consistent with previously published data (Andres et al., 2010).

The results are indicated by the open circles in Fig. 1B. A representative agarose gel showing unejected DNA from the capsid under different osmotic pressure conditions is included in the supplemental materials (Fig. S1); the length of DNA remaining in the capsid is seen to increase with increasing PEG concentration until ejection is completely suppressed at 16.8 atm. Because of the jump from 10.8 to 16.8 atm in our osmotic pressure measurements, it is possible that complete suppression occurs anywhere between these two pressures. But it is clear from the variation of ejection fraction with pressure for the set of measured points that 16.8 atm is an upper bound (and very close) to the value at which the suppression is complete. This is significant because, as we shall see in the following sections, ejection of the E proteins is largely *complete* at 16.8 atm in the presence of OmpA. For all samples, more than 80% of the capsids were triggered open as was confirmed by plating experiments in which the number of remaining plaque-forming units (PFUs) was counted after treatment with