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Is phage DNA "injected" into cells - biologists and physicists can agree

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Introduction

The Hershey and Chase experiment with phage T2 [1] led to the idea that phages inject their DNA via a syringe-like mechanism, an idea that was popularized by the earliest molecular genetics textbooks [e.g., 2, 3]. The source of energy for translocating DNA into the cell was suggested to be pressure inside the phage head due to the tightly packed phage genome. The aims of this review are to show that the "syringe model" is, by itself, incapable of explaining how a phage genome can enter an infected cell, and to summarize the sources of energy that can promote genome translocation. Actually, even a casual consideration of the syringe model reveals shortcomings: not all phage genomes contain double-stranded (ds) DNA (the source of pressure in the syringe model), many phages lack a tail long enough to span the cell envelope, and simple injection by a syringe model would therefore result in the phage genome being deposited in the extracellular medium or perhaps in the cell periplasm. Furthermore, only two years after the Hershey-Chase experiment was published, T5 was shown to follow a strategy different from T2 for transporting its genome into the cell [4]. This observation eventually led to the two-step mechanism for T5 DNA entry into the host cell [5]. Finally, the first kinetic measurements of phage genome ejection into cells revealed that DNA entered the cell at a constant rate [6–8], a result that is incompatible with a simple pressure-driven process. As discussed in more detail below, a pressure-based mechanism would predict a reduction in rate as the genome exits the capsid. Further, the rates of SP82 and T7 genome ejection *in vivo* were shown to be dependent on temperature, and the rate data could be fitted to an Arrhenius plot [6,9], demonstrating that entropy was not the driving force. In fact, cellular energy is required for all parts of the T7 genome to enter the cell. Thus the predictions of the "syringe model" and pressure-based phage genome ejection into cells failed completely in the first two cases examined experimentally! In contrast, the λ and T5 genomes can be ejected *in vitro* from virions into liposomes [10–12], and perhaps every phage worker has intentionally or otherwise "popped" a phage preparation where the genome has been released from the particle. We thus have a paradox; two phage genomes have been shown to be ejected into cells by a mechanism that is not controlled by pressure of the packaged DNA, whereas two others eject their genomes completely in an *in vitro* process where pressure of the packaged DNA is the driving force. Although our understanding of the physics of DNA condensation and constraints of DNA packaging into phage heads has improved enormously, this paradox between theory and experimental DNA ejection parameters *in vitro*, and *in vivo* observations has not been resolved.

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In this essay, we attempt to address some of the differences between the *in vivo* and *in vitro* studies.

Theoretical explanations of the ejection process have examined three basic effects. The first mathematical model describing phage dsDNA genome ejection was based on Brownian motion [13], but it was later concluded [14] that complete ejection of T4 DNA would take about 8 hours! Further theoretical work examined a second factor, osmotic pressure within the crowded environment of the capsid, which creates a force driving DNA translocation [14]. It was concluded that this pressure, about 30 atm, could theoretically eject DNA from the T4 virion at a rate of over 20 kbp/s (possibly as high as 1000 kbp/s), fast enough for an entire phage genome to enter the host cell within a few seconds. Recently, a number of models have been developed that take into account the osmotic pressure, electrostatic forces, entropy, and the bending stiffness of DNA, with the goal of computing this internal force throughout the translocation process [15–20]. There are implications for the rate of DNA translocation, but authors have avoided estimating this rate, due to uncertainties about friction and viscosity, which arguably may be the largest unknown factors in the ejection process. A third factor has been shown experimentally to play an important role for some phages; for example, specific molecular motors - including two different RNA polymerases (RNAP) - catalyze the translocation of the majority of the T7 genome into the cell [8,9,21,22].

In the following sections, we examine these processes in detail, emphasizing the features of each that might be generically applicable to all phages. We conclude by showing how one effect, the inrush of water due to the increased permeability at the site of phage attachment, could be generally responsible for at least part of phage genome translocation into cells.

Diffusion-based phage genome ejection

The simplest possible mechanism for DNA transfer from a bacteriophage into a host cell is diffusion, a random back-and-forth motion through the pore that continues until all the DNA has left the capsid. The process is as follows: the leading end of the DNA is released from within the virion and becomes free to move through the tail into the head. This initiation step, described by Stent [2] as the “uncorking reaction”, is an essential feature of any ejection mechanism. Second, random thermal agitation of the DNA causes it to move through the tail. A basic equation of diffusion relates the distance traveled x to the time t:

$$\langle x^2 \rangle = 2Dt,$$

where the angle brackets denote a statistical average. The diffusion constant D for any object is directly related to a mechanical quantity, the mobility μ, which describes how fast the object will move under a force F. Specifically,

$$\mu = v/F,$$

where v is the velocity at force F, and we have

$$\mu = \frac{D}{kT}.$$

Finally, after the DNA has diffused a distance equal to its contour length L, it enters the cytoplasm of the host cell. Within the cytoplasm, DNA has access to a volume 1000 times larger than the capsid, so it is unlikely that it will ever diffuse back to its original configuration. This simple model describes a straightforward way to estimate the translocation time: we just need to calculate μ and apply the equations to determine the time when

$$\langle x^2 \rangle \approx L^2.$$

To calculate μ, many authors have applied the equations of hydrodynamics to the motion of the DNA within a capsid. For example, μ can be estimated for DNA within a cylinder of dimensions similar to a typical phage tail. Such a model is based on physics that should be the same across many phage species, though its specific predictions will depend on the exact dimensions of the phage capsid.

However, a critical flaw of diffusive models is that they fail to take into account the non-random, directed forces that act on the DNA, which are typically on the scale of piconewtons (pN), arising from both pressure in the phage head and in the cell cytoplasm. To get a sense of the scale of such forces, suppose that a force is directed outward from the cytoplasm, and consider that diffusion can easily impart an energy of about 1 kT = 4 pN nm to the DNA. This energy is enough to translocate the genome by 4 nm = 12 bp. However, a typical genome is ~10–100 kb and many such pulses of energy, all by chance oriented in the same direction, would be necessary for its translocation into the cell. This is extremely unlikely to occur on the time scale of a phage growth cycle. Alternatively, if the non-random forces are directed in toward the cytoplasm, the DNA does not have to pass an energy barrier and can be pulled into the cell. In this case, fluctuations due to diffusion are very small relative to the overall motion of the DNA. In the following text we will discuss various sources of directed forces that are likely to be more important than diffusion in affecting the translocation process.

Pressure in the mature phage particle

The genome of most dsDNA bacteriophage is packed in the capsid to a density of about 500 mg/mL [23], which directly implies a counterion density of 1–3 molar and osmotic pressures of tens of atmospheres. This results in a force on a pN scale that could expel the genome from the virion. For several phage types, the counterions can be readily exchanged by dialysis, meaning that they are not tightly bound. However, the capsid of T4 and related phages contain polyamines at the concentration present in the host on which they were grown [24]. The T4 capsid is impermeable to polyamines, which normally neutralize about 40% of the T4 DNA-phosphate charges. However, T4 grows in polyamine-deficient cells and the resulting particles are infective [25]. Trapped small ions are therefore not a general feature of all phages and cannot be a necessary component of the DNA ejection process. Nevertheless, the fact that most dsDNA phages have similar densities of packaged DNA suggests that this feature is important in phage biology. Virions of *Pseudomonas acidovorans* øW-14 (morphologically related to T4) contain a genome packaged at sim;30% higher density, dependent on DNA containing the polyamine-modified base α-putrescinylthymine (putThy) [26]. About 25% of the DNA-phosphate charges can be neutralized by the putThy, and some neutralization is necessary because mutants partially defective in putThy synthesis package sim;11% less DNA than wild-type into virions. The normal upper limit of DNA packaging density may be due either to maintaining virion stability or to the maximum force generated during DNA packaging [27, 28]. Oversized (sim;52.5 kb) λ genomes are packaged with only 20% efficiency but are fully infective [29]. Conversely, both λ packaging and infectivity exhibit a minimum genome length (sim;36.5 kb), with infectivity being more sensitive. The packaging defect is due to the failure of terminase to recognize *cos* sequences efficiently [30], and those genomes that are packaged may not subsequently insert their DNA correctly partway down the tail. Whether these virions also contain sufficient pressure within the head to initiate genome ejection is not known. It is important to remember that the leading end of the dsDNA must be within or connected to the channel through the head-tail connector (portal) protein or inside the tail in a mature virion. If it were not, genome ejection would essentially never take place (think about spaghetti in a colander!).

Pressure-based phage genome ejection

The forces within the phage head under specific experimental conditions have been modeled and measured directly[14–20,31–36]. Calculations agree with *in vitro* measurements, implying that the forces ejecting the DNA are well-understood. How fast does the DNA exit a bacteriophage under these conditions? Several models of the rate of DNA translocation under pressure have been constructed. The models depend on an estimate of the mobility μ, as described above, which can be calculated using the established equations of hydrodynamics. Once μ is known, the velocity of translocation can be derived directly from the force expelling the DNA. Though the models differ in the details of the calculations and the assumptions made, their conclusions are similar. For example, in the simplest case where μ is a constant determined by friction within the phage tail, as the DNA density in the head decreases during ejection the driving force and translocation velocity also decreases. We thus expect the maximum translocation velocity at the beginning and a much lower velocity at the end. However, most models and *in vitro* experiments show that the pressure inside of a phage head decreases to zero before genome ejection has been completed. Consequently, the last part of the genome is predicted to exit the particle extremely slowly. If large polyvalent ions or soluble proteins remain in the capsid during DNA ejection, they would provide an osmotic pressure above that of the surrounding media that could complete efficient expulsion of the genome; in principle, the polyamines in a T4 virion could provide that pressure.

Recent single molecule experiments have measured the rate of DNA translocation *in vitro* under internal pressure alone. When λ is stimulated to eject its DNA *in vitro*, it exits the capsid at a rate of up to 60 kbp/s, and an entire 48.5 kbp genome has finished translocation within 1.5 s [37]. The translocation velocity does in fact decrease towards the end of ejection, but a computation of μ revealed that friction is greatest when the capsid is fully packed, and it drops to near zero as ejection is completed. This implies that the dominant source of friction is not passage of DNA through the λ tail but is present in the head, most likely due to DNA-DNA interactions. Regardless of whether the DNA is spooled into an orderly coaxial concentric spool, as in T7 and ε15 [38,39], a folded toroidal conformation as recently modeled for ø29 [40], or alternative arrangements there will be friction between a DNA segment exiting the head and those segments yet remaining. Clearly, as the head empties and the remaining DNA becomes less compact, those frictional forces will decrease. The λ DNA ejection result [37] is in overall agreement with a real-time experiment using T5 where, in part because of the nicks in a T5 genome, the ejection velocity could not be accurately resolved [41]. However, if the observation about friction in λ ejection is generalized to most phages, a reasonable assumption as the pressure of the packaged dsDNA is approximately the same in all phages, it is likely that other phages will show similar DNA ejection properties *in vitro* if subjected to a comparable assay.

However, the general implication that the force drops to zero during genome ejection *in vitro* presents a problem for phage infections *in vivo*. The internal osmotic pressure of the cell provides a resistive force to the entering phage genome. For example, the *E. coli* cytoplasm is thought to have an osmotic pressure of at least 2 atm, though it may vary with growth conditions and reach values as high as 15 atm [42–45]. *In vitro* experiments showed that 2 atm of external pressure is enough to hold about 15 kbp of λ DNA in the capsid [35]. Importantly, the addition of 1 mM spermine, which stabilizes the packaged virion DNA and should thus reduce internal pressure, to a λ particle completely suppresses DNA ejection *in vitro* [31], and *in vivo*, λ infection is effectively inhibited by 1 mM putrescine in the media [46]. Similarly, 10 mM spermidine reduced both the rate and extent of T5 DNA ejection *in vitro* [33]. In contrast, using an osmotic shock-resistant T4 mutant (the wild-type capsid is impermeable), it was shown that 10 mM putrescine not only allowed infection but it also suppressed the significant temporal delay of DNA injection into the cell caused by 50 μM proflavine [47]. Proflavine and other

intercalating dyes lengthen and stiffen the packaged DNA and should therefore increase the internal pressure. It is not obvious how to reconcile these data with different phages in terms of a general model for DNA ejection from a virion, but pressure-based models of phage DNA ejection *in vivo* will have to consider environmental conditions, capsid permeability, and packaged DNA structure in order to be generally applicable. These problems notwithstanding, as we have discussed above, the pressure associated with DNA packaged in the phage head is insufficient to completely eject a genome into a cell and diffusion is insufficient to internalize a significant length of DNA against the internal osmotic force of the cell cytoplasm. This conclusion demands that, for all phages, there must be an additional source of force driving, or at least completing, genome translocation.

Transport by specific proteins

The most compelling experimental evidence against an internal pressure mechanism providing the driving force for phage DNA ejection comes from real time measurements of T7 genome internalization [7–9]. Each step of the normally tripartite ejection process translocates DNA at a characteristic constant rate. DNA translocation from a T7 particle *in vivo* proceeds at least 10 times more slowly than from λ [48] and about 1000 times more slowly than from λ *in vitro* [37]. Instead of using pressure in the T7 head, the experiments show that most of the T7 genome is actively translocated by *E. coli* and then T7 RNAP at approximately their normal rates of transcription *in vivo*. When a transcribing RNAP approaches the cell membrane, the force usually thought to move the enzyme (plus associated mRNA, ribosomes and nascent proteins) along a template can be applied equally well to the template. This results in transcription pulling the phage genome into the cell. In fact, *E. coli* RNAP exerts a force of about 20 pN during transcription [49], which is likely to be sufficient to overcome the internal osmotic pressure of a cell without causing the rate of transcription to slow. Multiple copies of RNAP transcribing the same DNA molecule might even work together to create even higher translocation forces [50,51]. Even the leading end of the T7 genome is brought into the cell at a rate that is (using mutant virions that do not require transcription for genome internalization) constant over the entire 40 kb genome [9]; this process also has the properties of an enzyme-catalyzed reaction. In addition, N4 also depends on transcription by RNAPs for most genome translocation [52], and the distal part of the ø29 has been shown to be pulled into the cell [53]. These experiments all suggest that specific energy-requiring motor proteins within the cytoplasm are responsible for active translocation for at least part of the phage genome. A variation of this idea is that tight binding of DNA to intracellular proteins, even in the absence of enzyme activity along the DNA, may cause translocation via a "Brownian ratchet" mechanism [15,20]. In this case the energy input results simply from protein-DNA binding. However, the bacterial chromosome would effectively compete with non-enzymatic, sequence non-specific, DNA-binding proteins like HU and prevent the latter from directing a fast internalization of most phage genomes. One exception to this generalization is phage T5, which completely degrades the host chromosome after internalization of the leading 8% of its genome (first-step transfer, FST) [5]. Non-sequence-specific DNA-binding proteins could then translocate the distal 110 kb (second-step transfer, SST) of the T5 genome. Interestingly, if the capsid is stripped from the infecting particle after FST, the 110 kb naked SST DNA was still internalized, and at a rate not substantially different from a normal infection [54]. This is the clearest example that pressure within the phage head is not essential for DNA internalization by the cell!

Under the protein-catalyzed model of genome translocation, different phages will presumably utilize different proteins for translocation, and these proteins will work in different ways and at different rates. Does every phage need to include its own unique and possibly exotic molecular motor to internalize the majority of its genome? This would be very unsatisfying, because we would not be able derive general principles to guide models. Transcription cannot

be a general mechanism for translocation of the bulk of a phage genome because many phage genomes are circularly permuted, which precludes promoters (or comparable sequence-specific DNA-binding proteins) being appropriately positioned near the leading end of each genome. This does not preclude transcription (or comparable processes) being used for the later stages of genome internalization) but, it has been shown that the λ genome is completely and rapidly internalized in the absence of transcription [48]. Using a single molecule approach, the timescale of λ genome ejection *in vivo* is anyway so fast, possibly as high as 10 kb/s (T. Winther and R. Phillips, personal communication), much faster than *E. coli* RNAP synthesizes mRNA at 37°C (40–50 bp/s) [55]. Only FtsK, a motor that translocates DNA at 7 kb/sec [56], approaches the required speed for rapid genome internalization. However, binding of FtsK to DNA is also highly sequence-specific [57] and its recognition site is not found on currently sequenced phage genomes, precluding its use as a general mechanism for phage DNA translocation into cells.

The use of cellular molecular motors to transport DNA into the cell cytoplasm also necessitates a prior step where the leading end of the genome is made accessible to those motors. In principle, that step could be driven by pressure within the phage head, but in the one system examined to date this is not the case. The leading 850 bp of the 40 kb T7 genome is normally internalized by the virion proteins gp15 and gp16, which are ejected into the cell before any DNA [8, 58, C. Chang and IJM, unpublished]. Virions containing a mutant gp16 internalize the entire genome in the absence of transcription at a constant rate that varies with temperature [8,9,59]. The process requires the membrane potential and has the properties of being enzyme-catalyzed. It was concluded that the virion proteins gp15 and gp16 form a molecular motor that ratchets the phage genome into the cell. It is not known whether a comparable process operates in phages other than the T7 family, but it is clear that the entire λ and T5 genomes can completely enter an energy-poisoned cell [60,61], and the membrane potential may be important only for penetration of the T4 tail tube through the cytoplasmic membrane rather than DNA translocation itself [62].

If a membrane potential is not obligatory for DNA translocation, it is hard to see what else could possibly provide a sufficient force to overcome the internal osmotic pressure of the cytoplasm. In the next section we discuss and provide a quantitative analysis to a recently proposed theoretical solution [63]. The model also provides a mechanism for transport of ssDNA and multipartite genomes and of multiple protein molecules from an infecting virion into the cell. Likely because of the emphasis on DNA rather than protein being the genetic material, most discussions on the Hershey-Chase experiment [1] neglect to mention that proteins are also ejected from a phage virion into the infected cell. One of the first descriptions was by Hershey himself [64], only three years after his seminal publication [1]. It is therefore not widely appreciated that most, if not all, phages do eject proteins into the cell. Ejection may take place before DNA, as is the case with T7 [58], attached to the ends of the genome as, e.g., T4 and ø29 [65,66], or in principle after complete DNA ejection. It is not likely that the channel from the phage head into the cell cytoplasm can accommodate DNA and protein simultaneously. The ejection of T7 proteins could be driven by forces associated with the packaged genome, although how the orderly exit of multiple protein species is achieved in order to form the extensible T7 tail [67] and to establish the DNA translocation machinery is unknown. In principle, if proteins do not leave the phage head until after the DNA, as discussed above they could provide the pressure for complete genome ejection. But how are those same proteins then ejected into the cell?

Osmotic gradients and phage genome injection into the cell

The preceding models of phage genome ejection were limited by the small scale of the predicted forces, an inability to explain how DNA is imported against cytoplasmic osmotic pressure, and

a lack of generality. It would be desirable to find a model that can explain translocation of an entire phage genome, at a minimum that part of a genome that cannot be transported using the pressure associated with packaged DNA. The model should also ideally rely on an effect that should be present for all phage/host combinations. The transcription-based mode of genome internalization used by T7 and N4, for example, could then be considered exceptions to the general rule. One component that is present in all infected cells is water, and here we turn to water as a potential source of energy for translocating a phage genome.

It is important to understand the role that osmotic pressure plays in bacterial cell growth. For the following discussion, we consider the osmotic pressure Π to just be a function of the concentrations of the various solutes, which can be approximated by the ideal gas formula $\Pi = nkT$. When solutions with an osmotic pressure difference Π are allowed to come to equilibrium across a semipermeable membrane, a hydrostatic pressure $P = \Pi$ will be built up. For example, if dialysis tubing is filled with a PEG solution and inserted into pure water, water will rush in, building up hydrostatic pressure until this condition is reached several minutes later. In the case of a bacterium, where we will assume Π is about 2 atm, the continuous influx of water is absolutely necessary for the continued growth of the cell, so a growing cell always exists in a state with $P < \Pi$. In addition to Π allowing water influx, *P* inflates the cell, giving it a certain amount of structure and rigidity. Water influx is facilitated by specific channels in the cytoplasmic membrane, such as *E. coli* AqpZ, and many phage infections themselves are thought to open a transient channel that has a significant permeability to water [68].

The equations describing water flow around DNA have already been described [14]. However, the authors assumed that water flows out of the cell as the DNA enters, providing resistance to DNA movement into the cell. However, based on the preceding discussion, we see that when $P < \Pi$ – the usual situation for growing bacteria – water is expected to flow into the cell. In the case of phage infection, water can flow into the phage head, through the tail and into the cell. As water flows past the DNA, it will apply a hydrodynamic force that could pull DNA into the cell. This idea provides a possible mechanism of DNA translocation.

Several geometric quantities are relevant for calculating the force on the incoming DNA due to the flow of water. Using λ as the example, tail length is approximately $L = 100$ nm. In order to simplify calculations we assume that the tail is a smooth cylindrical tube with inner radius $R = 2.0$ nm, a value consistent with electron micrographs of many phage virions. The tube diameter can accommodate the double-stranded DNA, itself assumed to be a cylinder of radius $r_{DNA} = 1$ nm, plus up to three layers of water or a combination of counterions plus water. For water flowing between concentric cylinders, we compute a force due to the flow given by:

$$F=\pi r_{\mathrm{DNA}}\frac{\Pi-P}{2}\left(\frac{R^2-r_{\mathrm{DNA}}^2}{r_{\mathrm{DNA}}\log R/r_{\mathrm{DNA}}}-2r_{\mathrm{DNA}}\right)-\frac{v\cdot 2\pi\eta L}{\log R/r_{\mathrm{DNA}}}.$$

Here, *v* represents the DNA translocation velocity and η is the viscosity of water. For water at 37°C, we have $\eta = 0.7\text{x}10^{-3}$ Ns/m^2. Notice that the above equation consists of two terms: one that is linear in the pressure difference $\Pi - P$ and one that that is linear in *v*. Using the numbers given for λ above, we find:

$$F=3.7\mathrm{nm}^2\cdot(\Pi-P)-2.2\times10^{-4}\mathrm{pN\,s/kbp}\cdot v.$$

To estimate $\Pi - P$, we need to use information about a typical bacterial cell. Let us consider *E. coli*, with a typical volume of 0.4 μm^3 = 4 x 10^8 nm^3, growing with a doubling time of 1200 s. As the cell grows in size, water flows in at a rate:

$$\Phi = 4 \times 10^8 \text{nm}^3 / 1200 \text{ s} = 3.3 \times 10^5 \text{nm}^3/\text{s}.$$

The water channel AqpZ is thought responsible for most of the permeability of the cell to water, an idea that was tested by subjecting cells to transient osmotic shocks [69]. A 1 mosM (2.6 pN/nm^2) shock caused AqpZ$^+$ cells to begin shrinking at 0.2 cell volumes/15 s = 5.3 x 10^6 nm^3 /s, while AqpZ$^-$ cells shrank about ten times slower. This means that AqpZ is indeed responsible for most of the water transport across the cell membrane, and we can now estimate the relationship between $\Pi - P$ and Φ:

$$\Pi - P \approx 5 \times 10^{-7} \text{pN nm}^{-5} \text{s}^{-1} \cdot \Phi.$$

If this relationship holds, we can compute $\Pi - P$ under conditions of rapid growth:

$$\Pi - P = 0.16 \text{ pN/nm}^2 = 1.6 \text{ atm},$$

implying that P is only 0.4 atm during rapid growth.

What this pressure differential means is that at ν = 0, the influx of water produces a force F = 0.6 pN. In the language of molecular motors, this is the *stall force*, the maximum force that can be applied against the translocating DNA without it being pushed back into the capsid. We can also see that F = 0 when ν= 2700 kb/s: this velocity is the maximum possible translocation rate in the absence of any opposing force. This high velocity would cause complete translocation within 20 ms. In fact, λ DNA has been shown to pass through artificial nanopores in several ms [70]; however, when exiting a phage capsid, friction from the highly condensed DNA in the head would slow the process. Here we are only considering friction from water in the tail.

In our example of λ DNA translocation into the cell, the opposing pressure is given by P = 0.04 pN/nm^2, leading to an opposing force on the DNA of πr^2=0.13 pN, where r =1 nm is the radius of DNA. The opposing force is lower than the stall force, implying that influx of water along the osmotic gradient can be sufficient to overpower the internal pressure of the cell and internalize the infecting λ genome by hydrodynamic drag.

Our example clearly contains many assumptions, not least of which are that the tail tube interior is a smooth cylinder of radius 2.0 nm. If the radius is reduced to 1.3 nm, the DNA counterions would then be contained within the major and minor grooves, and only a single layer of water molecules would fit between the DNA and the tube wall. In this case, the hydrodynamic drag on the DNA due to water influx into the cell is less than half the opposing forces of the cell cytoplasm and cannot provide the sole mechanism of genome internalization. Without an atomic model of a phage tail it is not yet possible to compute the real situation, where the channel through which DNA is translocated has a variable diameter, and where interactions between the amino acids of the tail and the phosphodiester backbone of the DNA may be significant. In fact, detailed structures of connector or portal proteins show that the channel leading from the head to the tail does contain constrictions [39,71–74], and further analyses are premature before knowing whether the tail also contains such constrictions. Nevertheless, the primary aim of the above calculations is to show that hydrodynamic forces may be very important in the overall pross of translocating an infecting phage genome into the cell.

In the above discussion we also assumed that the osmotic pressure in the cell was sim; 2 atm, but experimental measurements vary widely [42–45]. Different experimental methods and growth conditions are at the root of the variation, and cells respond to increases in the external osmolarity by shrinking the cytoplasm and synthesizing osmoprotectants in order to maintain appropriate turgor pressure. However, if internal osmotic pressures are actually at the high end

of the range measured, little of a phage genome could be internalized by the pressure of the DNA packaged in the virion. Regardless of absolute values, there is some agreement that the internal osmotic pressure drops as cells approach and then enter stationary phase. The reduction in cellular osmotic pressure in stationary phase cells would reduce the force opposing phage genome internalization, but it would also reduce the hydrodynamic forces acting on the entering DNA. The net effect is hard to quantify. Few studies have been conducted on the efficiency of phage infecting stationary phase cells, and it should be remembered that the physiology of overnight broth cultures is not the same as cultures starved for all nutrients. However, it is worth noting that T7 makes a substantial burst in starved cells whereas T4 does not [75]. Furthermore, T7 and T3 make plaques on L-form *E. coli*, cells growing in osmotically balanced media in the presence of penicillin [76], where only a small osmotic difference can exist between the cytoplasm and the external media. Under the same conditions, T1, T4, T5, and λ were unable to form plaques. These observations can be rationalized because aside from requiring cells to have a membrane potential for T1 irreversible adsorption [77] and T4 tail tube penetration of the cytoplasm [62], genome ejection of these four phages is thought to depend on non-enzymatic processes. In contrast, the T7-like phages use energy-requiring molecular motors to internalize their genome, and can therefore be insensitive to any osmotic gradient between the cell cytoplasm and the extracellular medium.

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