Dynamics of DNA Ejection from Bacteriophage

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ABSTRACT   The ejection of DNA from a bacterial virus (i.e., phage) into its host cell is a biologically important example of the translocation of a macromolecular chain along its length through a membrane. The simplest mechanism for this motion is diffusion, but in the case of phage ejection a significant driving force derives from the high degree of stress to which the DNA is subjected in the viral capsid. The translocation is further sped up by the ratcheting and entropic forces associated with proteins that bind to the viral DNA in the host cell cytoplasm. We formulate a generalized diffusion equation that includes these various pushing and pulling effects and make estimates of the corresponding speedups in the overall translocation process. Stress in the capsid is the dominant factor throughout early ejection, with the pull due to binding particles taking over at later stages. Confinement effects are also investigated, in the case where the phage injects its DNA into a volume comparable to the capsid size. Our results suggest a series of in vitro experiments involving the ejection of DNA into vesicles filled with varying amounts of binding proteins from phage whose state of stress is controlled by ambient salt conditions or by tuning genome length.

INTRODUCTION

A crucial first step in the life cycle of most bacterial viruses (i.e., phage) involves binding of the virion to a receptor protein in the host cell membrane followed by injection of the phage DNA. The viral genome is typically $\sim 10\ \mu$m long, and its translocation from outside to inside the host cell is accomplished over times that vary from seconds to minutes. The wide range of mechanisms responsible for injection of phage genomes has recently been systematically reviewed (1–3), including many references to the last few decades of relevant literature. In this article, we formulate a general theory of chain translocation that takes into account many of the physical phenomena involved in actual phage life cycles. These phenomena include: diffusion of the DNA chain along its length; driving forces due to stress on the DNA inside the viral capsid; resisting forces associated with osmotic pressure in the host cell; cell confinement effects that constrain the injected chain; and ratcheting and pulling forces associated with DNA-binding proteins in the host cell cytoplasm.

Considerable effort has been focused on the energetics of packaging and ejecting DNA in phage. In particular, theoretical work (4–11) has shown that the dominant source of stress on the DNA in the capsid results from strong repulsive interactions between neighboring portions of double helix that are confined at average interaxial spacings as small as 2.5 nm. Another major contribution comes from the bending stress that arises from the capsid radius being smaller than the DNA persistence length. The force needed to package the genome against this resistance is provided by a virally encoded motor protein that pushes in the DNA along its length. Recent laser tweezer measurements (12) have confirmed that this force increases progressively as packaging proceeds, i.e.,

as the chain becomes more crowded and bent, reaching values as large as 50 pN upon completion. Conversely, the force ejecting the DNA upon binding of the phage to its membrane receptor has been shown (13,14) to decrease monotonically from tens of picoNewtons to zero as crowding and bending stress are progressively relieved. In this article, we consider the dynamics of phage ejection and attempt to distinguish the relative importance of these large, varying, internal forces and the binding particles in the external solution (bacterial cytoplasm).

It is useful at the outset to consider the simple diffusion limit of the translocation process. More explicitly, consider the case in which a chain is threaded through a hole in a membrane dividing one solution from another. If the chain is free, i.e., in the absence of pushing or pulling forces and of binding particles, it will simply diffuse along its length, experiencing a friction associated with its passage through the membrane and the viscosity of the solution. The time required for its translocation from, say, the left to the right will be $L^2/2D = \tau_d$, where L is the length of the chain and D is its effective translational diffusion coefficient.

Suppose now that particles are added to the right-hand solution, which binds irreversibly to the chain at regularly spaced sites as soon as they diffuse into the solution. Then, if s is the spacing between these binding sites, the diffusion of the chain will be ratcheted each time another length s has entered the solution (15–17), corresponding to the fact that the chain cannot move backward through the hole at a site where a particle is bound. Accordingly, the time it takes for the entire chain to appear on the right is simply given by $s^2/2D$—the time required for diffusion between a pair of neighboring binding sites—times the total number of sites, L/s. It follows that the overall translocation time in the presence of perfect ratcheting is reduced by a factor of s/L over that for free diffusion. When the binding of particles is

reversible—they do not remain bound indefinitely, thereby allowing some sites to diffuse backward through the hole— the translocation time is increased by a factor of $(1 + 2K)$ compared to perfect ratcheting, where K is the ratio of off- and on-rates for particle binding (15,16). Finally, note that the ideal ratcheting time of $Ls/2D$ corresponds to a velocity of $2D/s$ and hence, by the Stokes-Einstein relation, to a force of $2\, k_BT/s$ pulling the chain into the particle-containing solution (18).

When the particle binding is reversible, however, it turns out that there can be a different mechanism from the ratcheting dynamics, one that can significantly shorten the translocation time below $Ls/2D = \tau_{\mathrm{idealratchet}}$. This effect requires that the diffusive motion of the chain is slow enough and is due to the fact that the entropy of reversibly bound particles increases when there is more chain for them to explore. As a result, the entropy is an increasing function of chain length available in the right-hand solution. Indeed, in the limit of fully equili- brated binding, the system is equivalent to a one-dimensional Langmuir adsorption problem (18,19) (P. G. de Gennes, private communication, 2002; see also Reversible Force from the Binding Proteins, this article) . More explicitly, the one- dimensional Langmuir pressure can be written in the form $P_{1D} = (k_BT/s) \ln \left\{ 1 + \exp \left((\epsilon + \mu)/k_BT \right) \right\}$, where $\epsilon > 0$ is the energy lowering of the adsorbing particles upon binding and μ is their chemical potential in solution. Note that in the limit of large binding energy $((\epsilon + \mu)/k_BT \gg 1)$, this pressure reduces simply to $(\epsilon + \mu)/s$, which—because pressure is force in a one-dimensional system—can be directly interpreted as the force pulling on the chain due to the reversible binding of particles. Note further, in the large binding energy limit, that this force is necessarily large compared to the ideal ratcheting force, $2\, k_BT/s$ (18).

Ambjornsson and Metzler (19) have recently clarified the various timescales that determine the different regimes of chain translocation in the presence of chaperones, i.e., bind- ing particles. The first, τ_0, is the time needed for the chain to diffuse a distance of order s, the separation between binding sites. The second and third are τ_{occ} and τ_{unocc}, the char- acteristic times that a binding site remains occupied and un- occupied, respectively. The values τ_{occ} and τ_{unocc} are related by the equilibrium relation,

$$\frac{\tau_{\mathrm{occ}}}{\tau_{\mathrm{unocc}}} = \exp\left(\frac{\epsilon + \mu}{k_BT} \right). \tag{1}$$

Finally, τ_{unocc} can be approximated by the typical time it takes for a particle to diffuse a distance of order $R(\simeq c_0^{-1/3})$ between binding free particles,

$$\tau_{\mathrm{unocc}} = \frac{R^2}{2D_0} \simeq \frac{1}{D_0 c_0^{2/3}}, \tag{2}$$

where D_0 is the diffusion coefficient of the particles, and c_0 is their number density. One can then distinguish between three different regimes:

1. Diffusive regime: $\tau_0 \ll \tau_{\mathrm{unocc}}, \tau_{\mathrm{occ}}$. Here, the binding particles are irrelevant to the chain translocation because the chain diffuses its full length in a time too short for the particles to bind.
2. Irreversible binding regime: $\tau_{\mathrm{unocc}} \ll \tau_0 \ll \tau_{\mathrm{occ}}$. Here, particles bind essentially irreversibly on a timescale short compared to the time it takes for the chain to diffuse a distance between binding sites. We shall refer to this as the ratcheting regime.
3. Reversible binding regime: $\tau_{\mathrm{unocc}}, \tau_{\mathrm{occ}}, \ll \tau_0$. Here, diffusion of the chain along its length is slow compared to the time required for an on/off equilibrium of the bind- ing particles to be achieved. We shall refer to this as the Langmuir regime.

It is also important to clarify some relevant length scales involved in the problem. Specifically, we distinguish be- tween two extremes of how the separation, s, between bind- ing sites compares with the range, δ, of the attractive interaction between binding particle and the chain. Pure and perfect ratcheting will arise when $\tau_{\mathrm{unocc}} \ll \tau_0 \ll \tau_{\mathrm{occ}}$, in- dependent of the relative values of δ and s. Imperfect ratcheting will arise when $\tau_{\mathrm{unocc}}, \tau_{\mathrm{occ}}, \ll \tau_0$, but $\delta \ll s$. The translocation time for the imperfect ratchet is higher than the perfect ratchet by a factor of $(1 + 2K)$. Finally, when $\tau_{\mathrm{unocc}}, \tau_{\mathrm{occ}}, \ll \tau_0$ and $\delta \approx s$, we will have a Langmuir force acting on the chain. Note that, if the binding free energy between DNA and the binding proteins is very large, then $K \ll 1$. Also, when the range of attraction δ is comparable to the spacing between the binding sites s, the reversible binding of the proteins will result in a Langmuir force on the DNA chain. In the rest of the article we use $K \ll 1$ and $\delta \approx s$. A schematic of the role of these various effects is shown in Fig. 1.

Before proceeding further, it is instructive to make some numerical estimates. Within this simple translocation model all timescales are naturally referenced to that for pure
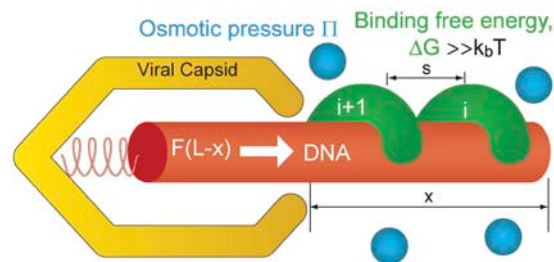

FIGURE 1 Schematic showing the various physical effects that assist bare diffusion in the process of phage DNA ejection. The DNA cross-section is not shown to scale: its diameter is 2–3 nm, as compared with a capsid radius that is 10 times larger. The spring denotes schematically the stored energy density resulting in a force F acting along the length $L-x$ of chain remaining in the capsid. The small spheres denote particles giving rise to an external (cytoplasmic) osmotic pressure Π_{osmotic}, and the green particles labeled i and $i + 1$ are successive binding particles. (The schematic and the model were inspired by Fig. 10.10 in (17).)

translational diffusion of a chain along its length, and hence to the diffusion coefficient D introduced earlier. In reality, however, the DNA ejection process is enormously more complicated, since the chain moving through the tail of the phage is feeling not only the friction associated with the few hydration layers surrounding it but also the viscous effects arising from interaction with the inner surface of the tail just nanometers away. Furthermore, this chain portion is connected to the lengths of chain inside the capsid and outside in the cell cytoplasm. The chain remaining inside the capsid moves by reptating through neighboring portions of still-packaged chain and/or by overall rotation of the packaged chain. All of these latter motions involve viscous dissipation that is insufficiently well-characterized to enable realistic estimates of diffusion timescales, even though one can distinguish between the different dependences on chain length for each of these dynamical processes (9,20). If the dominant source of dissipation is due to the friction/attraction between the DNA and the phage tail, the diffusion coefficient will be independent of the amount of DNA ejected (21). On the other hand, the diffusion coefficient D, in general, may depend on the amount of DNA ejected. To keep the matters simple, we assume that it is possible to define an effective diffusion coefficient D, and define the unit of time, $\tau_d = L^2/2D$. This way we can make predictions of how the ejection timescales with the genome length, for example, without knowing what the actual value of D is, although in the end it may be found that this picture of diffusion is too simple and a length-dependent diffusion coefficient will have to be involved.

A strong upper bound for D can be obtained by considering the part of the dissipation arising as the chain moves through the tail portion of the virus. Taking into account only the friction between the DNA and the fluid in the tail we have, for example (20,22), $\zeta = 2\pi \, l\eta/\ln(\Delta/d)$. Here ζ is the friction coefficient, l is the length of the tail, η is the viscosity of water, Δ is the inner diameter of the tail, and d is the diameter of the double-stranded DNA. Taking $l = 100$ nm, $\eta = 10^{-9}$ pN – s/nm^2, $\Delta = 4$ nm (23), and $d = 2$ nm, we find $\zeta = 9 \times 10^{-7}$ pN – s/nm and hence a diffusion coefficient ($D = k_B T/\zeta$) of 5×10^6 nm^2/s. For a typical phage genome length (L) of 10 μm, this in turn leads to a diffusional translocation time ($\tau_d = L^2/2D$) of ~10 s, not unlike ejection times measured for phage λ (24) (P. Grayson, private communication, 2005). Recall, however, that this estimate is based on a value for D that is a strong upper bound, because of all the viscous dissipation contributions that were neglected, suggesting that the actual unassisted diffusional time is likely several orders-of-magnitude larger than this 10-s estimate. Indeed, the outcome of the work presented below is that the translocation time is shortened beyond τ_d by several orders of magnitude by a combination of effects dominated by pressure in the capsid and binding particles in the external solution. This simple estimate provides us with an interesting insight into the dissipation mechanisms involved, and suggests two possibilities:

1. The friction of water (and hence, dissipation) is much larger at such short length scales.
2. As mentioned above, there are several other dissipation mechanisms, which are not taken into account.

The outline of the article is as follows. In the next section we include the effect of capsid pressure by formulating a Fokker-Planck description of translocation driven by a combination of diffusion and spatially varying force, i.e., a force pushing the chain from one side that depends on the length of chain remaining on that side (corresponding to the portion still in the capsid and hence experiencing stress due to crowding and bending). We evaluate the mean-first-passage-time (MFPT) for translocation of an arbitrary length and thereby calculate the length ejected as a function of time, using estimates of the spatially varying ejection force from recent theories of phage-packaging energetics. We find that the translocation times are 2–3 orders-of-magnitude faster than the diffusional time. We also treat the case of ejection into a volume comparable to the capsid size (mimicking, say, studies in which phage are made to eject into small vesicles that have been reconstituted with receptor protein (25,26)) and find the dependence of ejection time on the relative sizes of the phage capsid and the vesicle. In DNA Ejection in the Presence of DNA Binding Proteins, we treat the further speedup in translocation due to ideal ratcheting and the Langmuir force arising from the reversible particle binding, respectively. We find that the simple ratcheting effect is small compared to that arising from the entropic force of reversible particle binding. The effect of reversible particle binding decreases the translocation time by another order of magnitude beyond that due to capsid pressure effects. Finally, these particle binding effects are shown to be sufficient to work against resistance forces due to external (i.e., cytoplasmic) pressure. In the final section (Discussion and Conclusion), we conclude with a discussion of related work by others, of additional contributions to ejection dynamics that will be studied in future theoretical work (in particular, the effect of RNA polymerase acting on the ejected DNA), and of experiments planned to test the various predictions made in this work.

KINETICS OF EJECTION DRIVEN BY PACKAGING FORCE

As discussed in the Introduction, we focus here on a chain that has been confined in a viral capsid and is ejected from it through a hollow tail just big enough to accommodate its diameter. To elucidate the essentials of this ejection process, we describe the translocation of the chain as a diffusion-in-a-field problem (21,27,28). In this case, involving the translocation of a linear polymer along its length, the diffusion coordinate is a scalar, i.e., the length of chain x that has been ejected from the tail of the virus. The external field is described by the potential energy $U(x)$ that gives rise to the

force $F(x) = -dU(x)/dx$, pushing on the chain when a length x of it has been ejected. This force is due to the remaining chain length $L-x$ being confined inside the capsid and thereby subjected to strong self-repulsion (U_{rep}) and bending (U_{bend}). The corresponding potential $U(x) = U_{rep}(L-x) + U_{bend}(L-x)$ is the free energy calculated in recent theories of DNA packaging in viral capsids (7,8,11). This energy is seen to decrease dramatically as ejection proceeds (i.e., as x increases), and so does the magnitude of its slope that constitutes the driving force for ejection.

The one-dimensional dynamics of a diffusing particle in the presence of an external field is a classic problem in stochastic processes (29), and, as argued above, can be tailored to treat the translocation of phage DNA under the action of an ejection force $F(x) = -dU(x)/dx$. Accordingly, the probability $p(x, t)$ of finding a length x ejected at time t is given by the Fokker-Planck equation

$$\frac{\partial p(x,t)}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial p(x,t)}{\partial x} + \frac{D}{k_B T}\frac{\partial U(x)}{\partial x}p(x,t)\right). \quad (3)$$

As a part of this stochastic description of the translocation-under-a-force process, it is natural to define a mean-first-passage-time (MFPT), $t(x)$, which gives the average time it takes for a length x to be ejected in the presence of the external field $U(x)$, namely (30),

$$t(x) = \frac{1}{D}\int_0^x dx_1 \exp\left(-\frac{U(x_1)}{k_B T}\right)\int_{x_1}^x dx_2 \exp\left(\frac{U(x_2)}{k_B T}\right). \quad (4)$$

It is useful to consider several limits of this general equation, the first corresponding to the familiar case of no external field. From $U \equiv 0$, the integrals in Eq. 4 reduce trivially to $x^2/2D$, giving the expected diffusion time, $t(x) = x^2/2D$.

For the case of constant force, i.e., $U = -Fx +$ constant, the integrals in Eq. 4 can also be evaluated analytically, giving (16)

$$t_{Constant\,Force}(x) = \frac{x^2}{D}\frac{\exp[-\beta Fx] + \beta Fx - 1}{(\beta Fx)^2}. \quad (5)$$

Here we have written β for $1/k_B T$, and taken $F = -dU(x)/dx > 0$ to denote the constant force driving translocation of the chain to the right (see Fig. 1). In DNA Ejection in the Presence of DNA Binding Proteins, we will apply Eq. 5 locally, over each segment of length s associated with a binding site, to calculate the ideal ratcheting corrections to force-driven translocation. Note that simple and ratcheted diffusion are overwhelmed by force-driven translocation when $\beta FL \gg 1$ and $\beta Fs \gg 1$, respectively.

In the most general instance of spatially varying external field $U(x)$, as in the case of capsid-pressure-driven translocation, the integrals in Eq. 4 must be evaluated numerically. In this way we calculate $t(x)$ from Eq. 4 for the $U(x)$ determined from a recent treatment (7,11) of the packaging energetics in phage capsids. This provides a one-to-one correspondence between each successive time $t(x)$ and the fraction of chain ejected $x(t)/L$ at that instant.

In Purohit et al. (7,11), the shape of the λ-phage capsid is approximated as spherical, and the DNA inside the capsid is assumed to be organized in a hexagonally packed inverse-spool. The potential $U(x)$ is expressed as a combination of the bending energy and the repulsive interaction between the DNA strands, and is given by

$$U(x) = U_{rep}(L - x) + U_{bend}(L - x)$$
$$= \sqrt{3}F_0(L - x)(c^2 + cd)\exp(-d/c)$$
$$+ \frac{2\pi k_b T\xi}{\sqrt{3}d}\int_{R_{in}}^{R_{out}}\frac{N(r)}{r}dr. \quad (6)$$

The values F_0 and c are experimentally determined constants (31) describing the interaction between neighboring DNA strands, ξ is the persistence length of DNA, d is the interstrand spacing, R_{out} and R_{in} are the radius of the capsid and the inner radius of the DNA spool, respectively, and $N(r)$ is the number of hoops of DNA at a distance r from the spool axis. We are interested in finding the internal force on the phage genome as a function of genome length inside the capsid. We do so using Eq. 6 and simple geometrical constraints on the phage genome inside the capsid. The number of loops $N(r)$ in Eq. 6 is given by $z(r)/d$, where $z(r) = (R_{out}^2 - r^2)^{1/2}$ is the height of the capsid at distance r from the central axis of the DNA spool. The actual volume available for the DNA—$V(R_{in}, R_{out})$—can be related to the genome length $L-x$ in the capsid, and the interstrand spacing d, giving an expression for R_{in} in terms of d, R_{out}, and $L-x$. This relation can be substituted for R_{in} in Eq. 6, which then can be minimized with respect to d to give the equilibrium interstrand spacing as a function of the genome length $L-x$ inside the capsid. In this way we determine the total packing energy as a function of genome length inside the capsid ($L-x$) or as a function of the DNA length ejected x, i.e., $U(x)$. Using this result and Eq. 4, we can evaluate the MFPT, $t(x)$, for the DNA ejection in λ as a function of the length ejected. The corresponding fraction ejected, $x(t)/L$, is shown as a function of time in Fig. 2, with the label "no confinement"; note that time here is measured in units of L^2/D.

The value of D can be estimated on the basis of this simple model by the following procedure. The experiment by Novick and Baldeschwieler (24) showed that in a buffer containing 10 mM of Mg^{+2} it took ~50 s for phage λ to completely eject its genome. The values for F_0 and c in buffers containing Mg^{2+} have been measured (31). Since the values measured for 5 mM and 25 mM Mg^{2+} were not significantly different, we assume that the forces at 10 mM will be identical, i.e., $F_0 = 12{,}000$ pN/nm^2 and $c = 0.3$ nm. Using these values in Eq. 4 and numerically evaluating it for $x = L = 48{,}500 \times 0.34$ nm, we find the total time for λ to eject its genome of 48.5 kbp is $t \approx (10^5$ nm^2/D) s. Then, since this value is experimentally estimated to be ~50 s (24), we infer that $D \approx 10^3$nm^2/s. This is approximately three orders-of-magnitude smaller than the D estimated in Introduction, consistent with all the sources of dissipation that were left
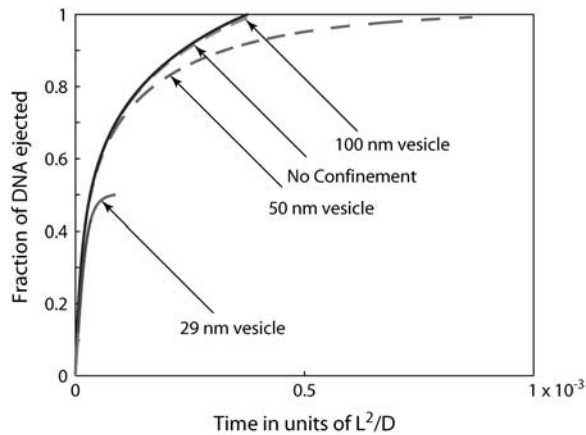
FIGURE 2 Ejection time for phage-λ injecting its genome into vesicles of radius 29, 50, and 100 nm. The capsid radius of the phage is 29 nm. It can be seen that the amount of DNA injection increases as the ratio of the vesicle radius to the capsid radius increases. On the timescale depicted here, there will be essentially no ejection due to pure diffusion (which takes place instead at times of order 1, in units of L^2/D).

out of that estimate. Note that once the parameter D has been fixed, there are no other free parameters in the model and hence all further deductions from the model are predictive. As will be shown below, the model developed thus far predicts the dynamics of in vitro ejection of phage DNA into lipid bilayer vesicles.

An interesting application of our estimates is to experiments in which viruses eject their DNA into lipid vesicles (2,24,26,32). Here lipid vesicles are reconstituted with the receptors recognized by the phage of interest, and then mixed with a solution of the phage. The phage binds to the receptor and ejects its DNA into the vesicle. We argue that the amount of DNA ejected into the vesicle and the corresponding time depends on the radius of the vesicle. In particular, if the vesicle has a radius comparable to that of the viral capsid, there will be a buildup of pressure inside the vesicle due to the ejected DNA. Ultimately, the ejection process will come to a halt when the force on the DNA from the capsid equals the force from the vesicle side—this can be thought of similarly from the free energy perspective as a free-energy minimizing configuration. Hence, the ejection will not, in general, be complete.

We can work out the ejection rate for this process as follows. If x is the length of genome ejected into the vesicle, we denote the free energies of the DNA inside the viral capsid and the vesicle by $U_{\text{capsid}}(L-x)$ and $U_{\text{vesicle}}(x)$, respectively. The total free energy will be given by

$$U(x) = U_{\text{capsid}}(L - x) + U_{\text{vesicle}}(x). \qquad (7)$$

As explained before, we already know $U_{\text{capsid}}(L-x)$ (see Eq. 6); the expression for $U_{\text{vesicle}}(x)$ can be obtained similarly by assuming that the vesicle is like a spherical capsid and the DNA configuration inside is similar to that inside the viral capsid. Our assumed structure for the DNA in the vesicle is a highly idealized model, though we note that electron microscopy on such vesicles demonstrates that DNA within them

can adapt to highly ordered configurations (26). In the limit where the vesicle radius is large compared to that of the phage capsid we will recover the free injection result (DNA ejecting from phage into the surrounding solution).

The injection process will stop when the total free energy reaches a minimum, i.e., the total force on the DNA is zero. The predicted time for DNA injection is given by Eq. 4. We have worked out the kinetics of the ejection for bacteriophage λ (radius ≈ 29 nm) ejecting its genome into vesicles of radius 29, 50, and 100 nm. The phage is taken to be suspended in a solution of Mg^{+2} ions, and similarly the vesicle, with concentration that approximately gives the same values for F_0 and c, as discussed earlier. This yields a prediction for the kinetics of injection for different vesicle radius. It can be seen from Fig. 2 that when the size of the vesicle is comparable to the capsid size there is only a partial ejection of the DNA. When the vesicle size is almost twice the size of the capsid, nearly the entire genome is ejected, except for the last part of the DNA, which takes extra time because of the resistance offered to it from the DNA inside the vesicle. Finally, when the vesicle is more than three times the size of the capsid, DNA gets completely ejected from the phage capsid as if there were no vesicle. It is interesting to note that in the initial stages of ejection, all the curves for various vesicle sizes fall on one another because there is no resistance to the injection, but as the ejection proceeds, each curve reflects a different resistance.

It is also possible that the arguments given above for in vitro ejection into vesicles could be relevant to thinking about ejection into the crowded environment of a bacterial cell (33,34). As a result of the crowding within the host bacterium, the viral DNA may be subject to confinement effects like those induced by vesicles.

DNA EJECTION IN THE PRESENCE OF DNA BINDING PROTEINS

The *Escherichia coli* cell has as many as 250 types of DNA binding proteins (35). Some fraction of these proteins likely binds either specifically or nonspecifically to the phage genome as it enters the host bacterium. Accordingly, we consider what happens if the phage DNA is swarmed with binding proteins upon its entry into the host cell. Depending on the binding on/off rates, binding site density, and the strength of binding, we have a corresponding speedup of the DNA injection into the bacterial cell, relative to the pure force-driven case. In this section we explore this effect and see how, in addition to the speedup, it helps the phage inject its DNA against the osmotic pressure in the host cell.

Throughout the following analysis of particle binding effects, we assume that the chain is stiff on length scales (e.g., tens of nanometers for double-stranded DNA genomes) large compared to the size of the relevant binding particles (typically a few nanometers). We also assume that the binding particles are comparable in size to the distance between

sites; for an estimate of Langmuir forces in the more general case of larger binding particles, see Ambjornsson and Metzler (19).

DNA ejection due to the ratchet action

Consider a scenario (discussed in the Introduction; $\tau_{\mathrm{occ}} \ll \tau_0 \ll \tau_{\mathrm{unocc}}$) in which host cell binding proteins irreversibly bind on to the DNA at a rate much faster than the translocation rate. In such a case, once a binding site is inside the cell, it is immediately occupied by a binding protein. If the protein stays bound long enough, compared to the translocation time, it will prevent thermal fluctuations from retracting the DNA back into the capsid. As a result, the DNA will diffuse only between consecutive binding sites, instead of along its complete length. Depending on the spacing between the consecutive sites, it will bring about a speedup in the translocation compared to when it is only force-driven (16).

For simplicity, we assume that the protein binding sites are uniformly distributed along the length of the genome. If the distance between the consecutive binding sites, s, is small compared to the genome length, i.e., $L \gg s$, we can assume that the internal force on the genome due to the packaged DNA is effectively constant while the DNA chain is diffusing between binding sites. In that case the MFPT, t_{i}, for the DNA to translocate the distance s between the binding sites $i-1$ and i, is simply given by Eq. 5, with x replaced by s, and F replaced by F_{i}. The internal force F is of course a varying function of ejected length x, but to a good approximation is constant over each interval of length s. The subscript i on the force F denotes this approximately constant force on the DNA chain when the translocation is taking place between the $i-1$ and i^{th} binding sites, i.e., when length $(i-1)s$ has been ejected.

The total translocation time for ejecting length x of the DNA is given by a sum over the MFPTs for all the sections of length s, along the length x ejected. The MFPT as a function of x is given by

$$t(x)_{\mathrm{Ratchet}+U(x)} = \sum_{\mathrm{i=1}}^{\mathrm{x/s}} t_{\mathrm{i}}(F_{\mathrm{i}})\big|_{\mathrm{Eq.\,5}}. \qquad (8)$$

The corresponding plot for the fraction ejected, $x(t)/L$, as a function of time is shown in Fig. 3 for $s = 20$ nm: the ratcheting reduces the injection time by half as compared to when the ejection results exclusively from the internal force. From Eq. 5 it can be seen that the time will decrease exponentially as the spacing s decreases. The important qualitative consequence of the ratchet is that it helps (see Binding Proteins Enable DNA Ejection against Osmotic Pressure) internalization of the complete phage genome against osmotic pressure, when internal force alone is insufficient.

Reversible force from the binding proteins

Consider another extreme scenario (also discussed in the Introduction; $\tau_{\mathrm{occ}}, \tau_{\mathrm{unocc}} \ll \tau_0$) where DNA injects into a
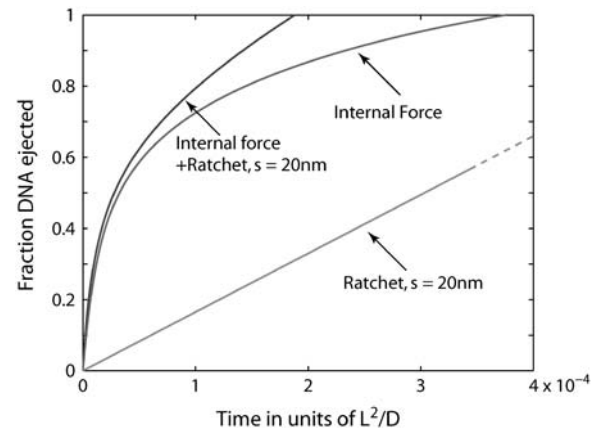

FIGURE 3 The fraction of DNA injected in phage λ as a function of time (in units of L^2/D) in the presence of binding particles that form a ratchet. The DNA injection purely due to the internal force is used as a benchmark, and the spacing between the binding sites $s = 20$ nm. It can be seen that the ratchet reduces the translocation time. The time required to internalize the genome solely by the ratcheting mechanism (see *lower, straight line*) is approximately twice the time taken for the purely internal force-driven mechanism.

reservoir of binding particles and the rate of translocation is slow compared to the time required for the particles to bind and unbind from the DNA. In this case, the binding proteins will come to equilibrium with the DNA. If, in addition, the range of attraction, δ, of the binding sites with the proteins is comparable to the spacing, s, between the binding sites, there will be an adsorption force pulling on the DNA, given by Zandi et al. (18) (P. G. de Gennes, private communication, 2002; see also Introduction),

$$F = \frac{k_{\mathrm{B}}T}{s}\ln\left\{1 + \exp\left(\frac{\epsilon+\mu}{k_{\mathrm{B}}T}\right)\right\}.$$
$$\approx \frac{\Delta G}{s}, \Delta G \gg k_{\mathrm{B}}T. \qquad (9)$$

Here μ is the chemical potential maintained by the reservoir of binding proteins, ϵ is the binding energy of the proteins with the DNA, and $\epsilon + \mu = \Delta G(> 0)$ is the binding free energy for the proteins. This adsorption force is the one-dimensional Langmuir pressure discussed in the Introduction. The origin of this equation can be seen by a simple derivation (36). Consider a stiff DNA segment of length l with binding sites separated by spacing s. The total number of binding sites on the piece of DNA is, hence, $l/s = M$. The DNA is surrounded by binding proteins at chemical potential μ. The grand partition function of the DNA and binding-proteins system is then given by

$$\Xi = \sum_{\mathrm{i=0}}^{\mathrm{M}} \frac{M!}{i!(M-i)!}\exp\left(\frac{i(\epsilon+\mu)}{k_{\mathrm{B}}T}\right),$$
$$= \left[1 + \exp\left(\frac{\epsilon+\mu}{k_{\mathrm{B}}T}\right)\right]^{\mathrm{M}}, \qquad (10)$$

and the grand free energy is

$$G = -k_{\mathrm{B}}T \ln \Xi = -M k_{\mathrm{B}}T \ln \left[1 + \exp\left(\frac{\epsilon + \mu}{k_{\mathrm{B}}T}\right) \right]. \quad (11)$$

It follows that the force acting on the DNA is

$$\begin{aligned} F &= -\frac{\partial G}{\partial l}, \\ &= -\frac{1}{s}\frac{\partial G}{\partial M}, \\ &= \frac{k_{\mathrm{B}}T}{s} \ln \left[1 + \exp\left(\frac{\epsilon + \mu}{k_{\mathrm{B}}T}\right) \right]. \end{aligned}$$

Note that, as mentioned in the Introduction, we assume that the range of attraction δ between the binding proteins and the DNA is comparable to the spacing s between the binding sites. If we further take the limit where $\epsilon + \mu = \Delta G \gg k_{\mathrm{B}}T$, we recover Eq. 9 for the force. This force has been observed by Zandi et al. (18) in their Brownian dynamics simulation, and is not the same as the ratcheting case because of the different timescales (as noted in the Introduction) involved in the two processes (19). Even though $\Delta G \gg 0$, in this case we have $\tau_0 \gg \tau_{\mathrm{occ}}, \tau_{\mathrm{unocc}}$, which is different than when ratcheting occurs ($\tau_{\mathrm{occ}} \gg \tau_0 \gg \tau_{\mathrm{unocc}}$). The Langmuir force is an entropic force acting on the DNA as opposed to the Brownian ratchet, which results only in the rectification of the motion.

To evaluate the MFPT we follow exactly the same process as in Kinetics of Ejection Driven by Packaging Force. The total force acting on the DNA is the sum of the internal pushing force and the external pulling Langmuir force. The total free energy involved with these two effects is given by $U(x) = U_{\mathrm{capsid}}(L-x) + \Delta G/s \times x$. The MFPT, $t(x)$, to inject x amount of DNA is then given by Eq. 4. We take a typical value of nonspecific DNA-protein binding free-energy of $\Delta G = 8\, k_{\mathrm{B}}T$ (37). The plot of DNA fraction ejected, x/L, against the corresponding ejection time, $t(x)$, is shown in Fig. 4 for $s = 20$
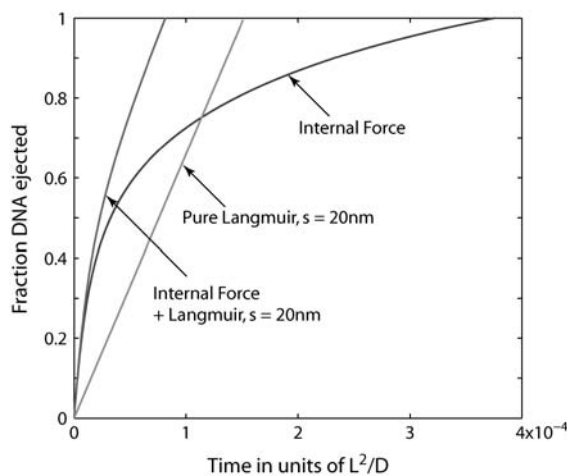

FIGURE 4 The fraction of DNA injected in phage λ in the presence of binding proteins that bind reversibly, as a function of time (in units of L^2/D.) The presence of reversible binding proteins results in a pulling Langmuir force (see text). This pulling force significantly enhances the DNA ejection rate over that of the purely force-driven mechanism, by almost a factor of 10.

nm. It can be seen that the Langmuir force speeds up the genome translocation by almost an order of magnitude. Not only that, but even if we do not have an internal force, this mechanism (see Fig. 4, *Pure Langmuir*) will inject the complete genome faster than the internal force-driven mechanism. This is because after ~50% ejection, the internal force begins to drop below the constant value of the Langmuir force. Indeed, from Fig. 4, we see that it is at an ejected fraction of ~0.5 that the slope of the internal-force curve drops below the constant slope (rate) of the Pure-Langmuir plot.

The two cases we described are really two extreme cases for the treatment of the role of DNA binding proteins. In reality, the rate of binding and the equilibration times may not be very fast (compared to translocation times) and the translocation rates would lie somewhere in between the rates evaluated in this section; for these cases it is necessary to treat the dynamical coupling between particle binding and chain diffusion (18).

Binding proteins enable DNA ejection against osmotic pressure

Due to macromolecular crowding (33), the *E. coli* bacterium has internal osmotic pressures of ~3 atm (38). The work of Evilevitch et al. (13) and Grayson et al. (14) showed for phage λ that the ejection process can be partially/completely inhibited by an application of osmotic pressure. Hence, it appears that if the phage were to rely entirely on the driving force due to the packaged DNA to eject its genome, the timescale for full ejection would be prohibitively long. On the other hand, since we know that the genome is completely internalized it seems likely that the particle-binding mechanisms described above may play a role in in-vivo DNA translocation. In this section we will see that the task can be accomplished by the Brownian ratchet and the one-dimensional Langmuir force mechanism discussed above in DNA Ejection Due to the Ratchet Action and Reversible Force from the Binding Proteins.

To see how the Brownian ratchet can internalize the genome against the osmotic pressure, we use the following procedure. If the osmotic pressure in the host cell is Π_{osmotic}, the resisting force acting on the DNA can be approximated (8,11) by $F_{\mathrm{osmotic}} = \Pi_{\mathrm{osmotic}} \pi R_{\mathrm{DNA}}^2$, where R_{DNA} is the radius of the DNA (~1 nm). For an osmotic pressure of 3 atm, the osmotic force is then estimated to be ~1 pN. We can now replace the term F in Eq. 5 with $F_i - F_{\mathrm{osmotic}}$ to evaluate the MFPT, t_i for the injection of the DNA segment between binding sites $i-1$ and i. This time t_i is then summed over all i, as in Eq. 8, to give the time $t(x)$ and hence $x(t)/L$. This fraction is plotted in Fig. 5 for the case of spacing $s = 20$ nm between binding sites, and for an osmotic pressure of 3 atm.

It can be seen from the figure (*bottom curve*) that the time required for internalizing the genome is comparable to the time it takes for phage to inject its genome purely by the internal force, when there is no osmotic pressure. The internal force for λ (data not shown) at ~50% DNA ejection is
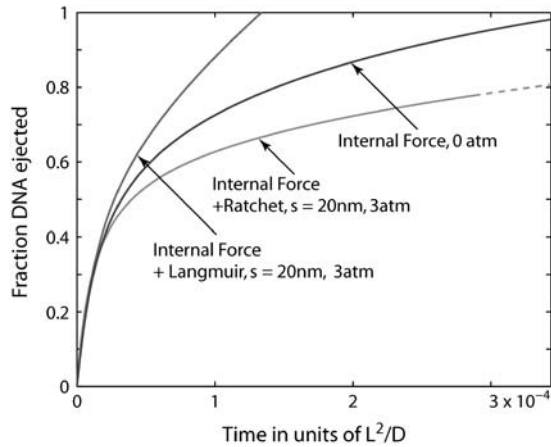
FIGURE 5   The fraction of DNA injected in phage λ as a function of time (in units of L^2/D) for the case in which there is a resistive force due to osmotic pressure. We compare the roles of the Langmuir force and the ratchet effect in ejecting the phage DNA against osmotic pressure. The spacing s is taken to be 20 nm and the osmotic pressure in the cell is $\sim$3 atm. It can be seen that the Langmuir force easily pulls the DNA against this pressure. The DNA translocation by the Brownian ratchet requires a much longer time, but it still succeeds in pulling out the genome at timescales not much longer than the ejection by internal force alone with zero osmotic pressure.

DISCUSSION AND CONCLUSION

This article addresses the problem of the kinetics of phage injection and the various mechanisms responsible for it. We make use of the available experimental data, existing models for phage packaging, and classical Fokker-Planck theory, to make predictions about translocation rates for phage DNA ejection. The key quantitative predictions described in this article are as follows.

Dependence of ejection rates on driving pressure

As shown in Fig. 2, the driving force due to the packaged DNA speeds up the ejection process by 2–3 orders of magnitude over free diffusion, and thus is a major contributor to the process of injection. Also, in the in vitro setting, the smaller the vesicle into which ejection occurs, the smaller the amount of DNA injected. In addition, for genomes of the same size, the time required for the ejection of the DNA is larger than when into a bigger vesicle. One of the simplest ways to control this driving force for ejection is by tuning genome length, and experiments are currently in progress (P. Grayson, private communication, 2005) to test these ideas.

Dependence of ejection rates on the presence of irreversible DNA-binding proteins

Ratcheting enhances the DNA ejection rate from the viral capsid. The speedup is minor when compared to internal force-driven ejection (see Fig. 3), but as seen from Fig. 5 it is sufficient to pull out the genome against osmotic pressures of up to 3 atm found inside the bacterial cell.

Dependence of ejection rates on the presence of reversible binding proteins

The reversible binding of proteins exerts a one-dimensional Langmuir force on the DNA. It can be seen from Fig. 4 that the presence of this phenomenon significantly enhances the DNA ejection rate beyond that due to pressure in the viral capsid. From Fig. 5 it is clear that this force is sufficient to efficiently internalize the phage genome against osmotic pressures of up to 3 atm in the bacterium.

We have several biological examples in mind when we treat these ejection mechanisms. In bacteriophage T5 the DNA injection occurs in two steps. The first step transfer, which involves ejection of $\sim$10% of the phage genome, is driven by the internal force (2). There is then a brief pause, when a protein is synthesized that is implicated in the degradation of the host chromosome, thereby freeing the large number of proteins that had been bound to it. These latter proteins are now possibly available for binding to the injected portion of the phage genome and for pulling the remaining DNA into the cell, via the ratcheting and Langmuir mechanisms. The calculations presented here call for a more

$\sim$1 pN, i.e., of the order of F_{osmotic}. It can be seen from Fig. 5 that the slope of the curve showing ejection in the presence of ratcheting and osmotic pressure starts decreasing at that percentage of ejection. The average force produced by a Brownian ratchet is $2\,k_BT/s \approx 0.4$ pN for $s = 20$ nm (16,18). At 60–70% ejection, the internal force is $\sim$0.5 pN; the total driving force is then $\sim$0.5 + 0.4 = 0.9 pN, which is almost the same as F_{osmotic}. This force hence works to eject the genome against the external osmotic force. When $\sim$15% of the genome is left in the phage capsid, the internal force is almost zero. At this point there is only a small amount of the genome still to be ejected and a small differential of $F_{\text{osmotic}} - F_{\text{ratchet}} \approx 0.5$ pN to be worked against. This is accomplished by the Brownian motion of the DNA.

Now, take the case when, as discussed in Reversible Force from the Binding Proteins, the Langmuir force acts on the DNA. To include the effect of the osmotic pressure we have to subtract the osmotic force $F_{\text{osmotic}} = \Pi_{\text{osmotic}} \pi R_{\text{DNA}}^2$ from the driving force $F_i + \Delta G/s$. The energy landscape associated with this force is simply $U(x) = U_{\text{capsid}}(L - x) + \Delta G/s \times x - \Pi_{\text{osmotic}} \pi R_{\text{DNA}}^2 \times x$. We use this energy in Eq. 4 to obtain the MFPT $t(x)$. From this expression, it can be seen that, as long as $(\Delta G/s - \Pi_{\text{osmotic}} \pi R_{\text{DNA}}^2) \geq 0$, we will always have DNA ejection proceeding faster than or similar to the rate for the purely force-driven nonosmotic pressure case. For the numbers we took in the preceding sections, $\Delta G/s = 8\,k_BT/s \approx 1.6$ pN—which is greater than $F_{\text{osmotic}} \approx 1$ pN. This implies that the phage would inject its genome faster than in the purely pressure-driven mechanism. The MFPT, $t(x)$, is plotted in Fig. 5, when $s = 20$ nm, $\Delta G = 8\,k_BT$, and $\Pi_{\text{osmotic}} = 3$ atm.

systematic experimental analysis of the extent to which proteins bind onto phage DNA as it enters the infected cell.

Similar ideas to those proposed here might also prove useful in those cases where the viral genome is translocated as a result of the binding of motor proteins, which themselves translocate along the DNA. One such example is the pulling force by the NTP-driven RNA polymerase (RNAP). RNAP is a very strong motor and can exert forces of up to 14 pN (39). As described by Molineux and co-authors (1,40), transcription by RNAP is the major mechanism for DNA injection from wild-type T7 into *E. coli* and is an intriguing additional active mechanism that is of great interest to treat theoretically as well.

In this work we have analyzed various effects of DNA translocation of internal capsid pressure and exterior (i.e., cytoplasmic) binding proteins that can be tested by a variety of in vitro experiments involving phage ejection kinetics into synthetic vesicles and through membranes formed over holes in planar partitions. In these ways one can separately control the capsid pressures (by varying salt concentrations or genome length, for example) and the nature and concentration of DNA-binding proteins inside the capsid or on the other side of the membrane. In addition, it will be important to examine the role of these various mechanisms in determining the kinetics of genome delivery in vivo.

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