Bacterial Translocation Ratchets: Shared Physical Principles with Different Molecular Implementations

How bacterial secretion systems bias Brownian motion for efficient translocation of macromolecules

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Secretion systems enable bacteria to import and secrete large macromolecules including DNA and proteins. While most components of these systems have been identified, the molecular mechanisms of macromolecular transport remain poorly understood. Recent findings suggest that various bacterial secretion systems make use of the translocation ratchet mechanism for transporting polymers across the cell envelope. Translocation ratchets are powered by chemical potential differences generated by concentration gradients of ions or molecules that are specific to the respective secretion systems. Bacteria employ these potential differences for biasing Brownian motion of the macromolecules within the conduits of the secretion systems. Candidates for this mechanism include DNA import by the type II secretion/type IV pilus system, DNA export by the type IV secretion system, and protein export by the type I secretion system. Here, we propose that these three secretion systems employ different molecular implementations of the translocation ratchet mechanism.

1. Introduction

For secreting and importing molecules, bacteria have evolved a variety of secretion systems.[1] In this article, we focus on secretion systems that support the transport of macromolecules, in particular the type I secretion (T1SS), type II secretion/type IV pilus (T2SS/T4PS), and type IV secretion (T4SS) systems. When transporting micrometer-sized polymers through nanometer-sized pores, the bacteria have to overcome physical barriers. First, the polymers must be threaded into the conduit of the secretion system. Second, the major part of the molecule must translocate the cell envelope efficiently.

Recent progress indicates that initial threading into the conduit of the respective secretion system is accomplished by ATP-consuming machines. However, for the subsequent translocation process through the outer membrane, ATP or proton motive force may not be available as energy sources. We propose that the translocation ratchet is an important part of the macromolecular secretion/import process; different molecular manifestations of chemical energy differences bias Brownian motion of the macromolecule within the conduit (Figure 1, Box 1).[2] We discuss experimental evidence supporting the idea that DNA and protein translocation by the T1SS, T2SS/T4PS, and T4SS employ different molecular implementations of the translocation ratchet mechanism. In particular, DNA uptake during transformation in gram-negative bacteria driven by the T4PS provides an example where chaperones residing within the periplasm bind to transforming DNA. Recent biophysical experiments provide strong evidence that periplasmic chaperones bias Brownian motion in the direction of uptake.[3] DNA secretion by the T4SS requires secretion of DNA-binding proteins into the cytoplasm by the host cell. Biophysical experiments in conjunction with cell biological approaches suggest that chaperones are secreted early during the infection cycle, generating a chemical potential difference for biasing DNA diffusion into the host.[4] RTX leukotoxins are secreted by a T1SS. Structural data together with functional analysis indicate that an intramolecular ratchet drives RTX secretion by Ca$^{2+}$-specific folding without the requirement for chaperones.[5]

2. DNA Uptake During Transformation and the Type II Secretion/Type IV Pilus System

Bacterial transformation is the uptake and inheritable integration of DNA from the environment. All naturally competent species characterized so far require proteins generating the type IV pilus (T4P) for DNA uptake during transformation.[6] The latter are similar to proteins forming the T2SS. Here, we will use the term “competence pilus” to describe the structure required
for binding and uptake of DNA. The only known exception is the T4SS-based *Helicobacter pylori* DNA uptake system.[6] T4P are polymerized by extraction of pilin monomers from the cytoplasmic membrane. In Gram negative organisms, these pili require the porin PilQ to cross the outer membrane and reach the extracellular space.[7] It is made of 12–14 PilQ subunits forming a pore that is 6.5 nm in diameter.[8] This diameter is sufficient for the passage of dsDNA (double-stranded DNA) which is 2.4 nm in diameter.[9] For all characterized competent gram-negative species, DNA uptake into the periplasm can be uncoupled from uptake into the cytoplasm. Uncoupling was shown genetically by deleting the genes encoding for cytoplasmic membrane transport[10] and by time-lapse microscopy revealing that outer and cytoplasmic transport are temporarily uncoupled.[11,12]

2.1. Competence Pili Are Likely to Thread DNA Into the DNA Uptake Complex

DNA uptake is initiated by binding of double-stranded DNA (dsDNA) to the competence pilus (Figure 2A). Interestingly, some species (including *Neisseriaceae* and *Haemophilus influenzae*) strongly select for self-DNA by preferentially taking up DNA with a recognition sequence (DNA Uptake Sequence, DUS).[13,14] For *Neisseriaceae*, specificity is achieved by binding to a minor pilin, ComP, that is most likely part of the competence pilus.[15,16] For *Vibrio cholerae*, there is no evidence for sequence-preference during DNA uptake.[17] The competence pilus is not only essential for DNA binding, but also for DNA uptake. In particular, the T4P retraction ATPase PilT is required for DNA uptake.[18] Extended T4P are dynamic polymers that continuously elongate and retract by polymerization and depolymerization of pilin subunits stored within the cytoplasmic membrane (Figure 2A).[19] The structural molecular basis for this trait has been elucidated recently.[20] T4P retraction generates considerable mechanical force and therefore T4P retraction was proposed to drive uptake of transforming DNA into the periplasm.[21–24] Recent experiments provide strong evidence against this hypothesis. First, the speed and maximum force of T4P retraction exceed the speed and maximum force of DNA uptake by orders of magnitude in *Neisseria gonorrhoeae*.[25] Second, experiments with T4P mutants

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**Figure 1.** The translocation ratchet. A) Polymer undergoing diffusive motion within a membrane pore. In the absence of directional bias, the probabilities of moving to the trans and cis side are equal. B) A difference in chemical potentials is generated by chaperones residing on the trans but not on the cis side. The chaperones bind to the polymer at a distance \( a \) and a dissociation constant \( K = k_{	ext{off}}/k_{	ext{on}} \). As a result, diffusion toward the cis side is inhibited, generating directional movement toward the trans side. C) Difference in ion concentrations between the cis and the trans side can support protein folding on the trans side, inhibiting backwards diffusion. D) Translocation time of a freely diffusing rod increasing with square of its length (black line). The diffusion constant was assumed to be \( D = 250 \text{nm}^2 \text{s}^{-1} \). Ratcheting with \( a = 1 \text{nm} \) (red) or \( a = 5 \text{nm} \) (orange) dramatically speeds up the translocation process.
Box 1. The translocation ratchet: A simple but efficient motor

In principle, thermal energy could power the process of macromolecular translocation through membrane pores or the conduit of secretion systems. Assuming that the molecule undergoes a one-dimensional random walk (Brownian motion within the pore) (Figure 1A), we can estimate the average translocation time by \( t = L^2/2D \), whereby \( L \) is the contour length of the polymer, and \( D \) is the diffusion constant of the polymer within the pore. Hence, the translocation time of a polymer increases with the square of its length. As a result, Brownian motion is an efficient transport mechanism for short molecules but becomes slow for long molecules (Figure 1D). Previous experiments of DNA translocation during bacterial transformation provide \( D \approx 250 \text{nm}^2 \text{s}^{-1} \).[3] Under these conditions, the uptake of a 10 kbp DNA fragment would take \( t \approx 6h \) (Figure 1D); a DNA uptake event would not be finished within one cell cycle.

However, chemical asymmetries between the cis and the trans side of the membrane may bias the polymer's random walk inside the pore in one direction, speeding up the translocation process. This idea has been formulated by Simon et al.[3] Chemical asymmetries can result from chaperones that bind to the polymer on the trans side (Figure 1B) or by gradients in ions or pH affecting the structure of the polymer (Figure 1C). Both chaperone binding and chain coiling or folding inhibit diffusion from the trans side to the cis side and generate net directional movement toward the trans side. The translocation time is then reduced to \( t = La/2D \) where \( a \) is the distance between two binding sites on the polymer (Figure 1B–D). In other words, the translocation time increases only proportionally to its length and not with the square of its length as in the case of free diffusion. Returning the aforementioned example, the uptake of the 10 kbp DNA fragment would take merely \( t \approx 7s \) for \( a = 1nm \) (Figure 1D). For this type of motor, Brownian motion generates movement and binding energy enables directional bias.

and naturally occurring antigenic pilus variants indicate that extended pili are not required for transformation.[26,27] Therefore, we propose that T4P retraction is important for the process of threading DNA through PilQ into the periplasm (Figure 2A), but the uptake of the major portion of DNA is driven by a translocation ratchet as described in the next paragraph.

2.2. Strong Evidence for Translocation Ratchet Driving DNA Uptake During Transformation

Subsequent to threading the DNA into the outer membrane pore, the major part of the DNA molecule has to translocate into the periplasm. Single molecule experiments have shown that fragments as long as 10 kbp or 3.4 μm can be imported without interruption.[3] Uptake of DNA from the environment (cis side) into the periplasm (trans side) occurs independent of transport through the inner membrane. However, no ATP is present within the periplasm and furthermore, no ion gradient is maintained over the outer membrane that may power molecular motors. Experiments have shown that 10 kbp DNA fragments enter the periplasm within a period of seconds,[3,17] rejecting unbiased diffusion as a mechanism for DNA uptake (Box 1). Very strong evidence has accumulated recently, supporting a translocation ratchet mechanism for DNA uptake wherein the periplasmic DNA-binding protein ComE(A) acts as the chaperone (Figures 1B and 2B).

The soluble, periplasmic DNA binding protein ComE(A) is essential for transformation and DNA uptake.[11,28,29] Experiments conducted with fluorescently labeled DNA revealed that ComE(A) was recruited within seconds to DNA entering the periplasm of \( N. \) \textit{gonorrhoeae} and \( V. \) \textit{cholerae}[11,28] and the concentration of ComE governs the capacity of the periplasm for DNA storage.[13] Various experiments indicated that the structural requirements for motor – DNA binding were less stringent compared to other DNA translocation motors, including the DNA packaging motor of bacteriophage \( \Phi 29 \).[10] First, replacing the native \( \text{comEA} \) of \( V. \) \textit{cholerae} by \( \text{comE} \) of \( N. \) \textit{gonorrhoeae} or even \( \text{comEA} \) of gram-positive \textit{Bacillus subtilis} supported DNA uptake.[28] Second, the motor supports import of dsDNA and single-stranded DNA (ssDNA) with similar kinetics, provided that the DUS is double-stranded.[31] The fact that ComE binds both ssDNA and dsDNA[30] is consistent with this observation. In line with these experiments, ComE(A) comprises a DNA-binding helix-hairpin-helix (HHH) motif that makes contact to DNA backbone phosphates and is thus not sequence-specific.[29,32] This peripheral interaction appears to be independent of DNA secondary structure, in contrast to the related, mostly sequence-specific helix-turn-helix motifs that bind inside the major groove of dsDNA.[33]

Recently, we obtained direct evidence for the role of ComE as a DNA binding chaperone of a translocation ratchet from single-molecule experiments measuring the kinetics of DNA import into the periplasm.[3] The concentration of ComE directly affects the velocity of DNA import, in agreement with theoretical predictions for a chaperone-assisted translocation ratchet.[14] Most importantly, Peskin et al. predicted an analytical expression for the velocity versus force relation of DNA uptake[35] (Box 2). Single molecule experiments showed that the force-velocity relationship of DNA uptake was in very good agreement with the theoretical predictions[3] (Box 2).

3. DNA Secretion Into Eukaryotic Cells and the Type IV Secretion System

Type IV secretion systems (T4SS) have the unique ability to mediate the translocation of DNA into eukaryotic target cells.[1] The Vir system in \textit{Agrobacterium tumefaciens} employs a T4SS to export tumor-inducing single-stranded Transfer (T-DNA) with a length of up to 150 kbp along with various effector proteins into the plant host.[16] The T4SS components form a macromolecular assembly spanning both membranes.[17,38] Moreover, the Vir
The T4SS that mediates effector protein and T-DNA secretion in \textit{A. tumefaciens} comprises 4 subassemblies: (i) The type IV coupling protein (T4CP) which is \textit{A. tumefaciens} VirD4; (ii) the inner membrane complex (IMC) that interacts with VirD4; (iii) the outer membrane complex (OMC) that interacts with the IMC and forms a channel that harbors the pilus; and (iv) the pilus that is required for the secretion of T-DNA and effector proteins.\[37\] EM and X-ray crystallography data have been combined to elucidate the architecture of the entire complex.\[40–43\] Moreover, a formaldehyde-crosslinking assay termed transfer DNA immunoprecipitation (TrIP) was employed to track the physical contacts of T-DNA on its way to the host cell.\[44\]

3.1. Initiation of DNA Secretion During Plant Infection Requires Multiple ATPases

In a first step, DNA-sequence-dependent accessory factors stimulate the nicking activity of relaxase VirD2.\[45\] A helicase unwinds the strands to yield ssDNA and the ssDNA-associated VirD4 binds to VirD4 via its N-terminal signal sequence.\[46,47\] The concerted activity of the hexameric ATPases VirD4, VirB11, and VirB4 is required for DNA translocation across the cell envelope via the T4SS.\[48,49\] As a transmembrane membrane protein and a hexameric ATPase, VirD4 has been proposed to thread ssDNA through its central annulus across the inner membrane (IM), similar to the translocation of dsDNA by the related ATPase FtsK.\[50,51\] (Figure 3A). VirB11 is structurally related to AAA\(^+\) ATPases and may assist translocation by unfolding of VirD2.\[52–54\] Further, VirB11 may liberate T-DNA from accessory factors by unfolding them. After traversing the IM, T-DNA interacts with the IMC and enters the channel formed by the OMC. Subsequently, T-DNA traverses the OM inside the pilus that is assembled within the channel.\[48,44\] VirD4, VirB11, and VirB4 are likely to energize transport through the channel. VirB10 functions as a signal transmitter relaying conformational changes in the cytoplasmic ATPases to the gating in the OM channels.\[1,55,56\] The pilus tip contacts receptors on the host cytoplasmic membrane and secreted T-DNA may enter the plant cell via membrane pores formed by previously injected VirE2 effector proteins.\[58,59\] Cytoplasmic VirE2 can then bind to start the ratcheting process.

3.2. Ratcheting by a Secreted Chaperone Is Likely to Support Import of T-DNA by Host Cells

Among the effector proteins required for a successful transfection with T-DNA is VirE2. VirE2 is the most abundant effector protein inside plant cells during an \textit{Agrobacterium} infection and has been shown to bind ssDNA to form molecular complexes in vitro.\[60–62\] Moreover, plant cells recombinantly expressing VirE2 can be transformed by T-DNA from VirE2 deletion strains.\[63\] Grange et al. proposed that VirE2 was an effector that is transported into the host cytoplasm at an early stage to actively pull the T-DNA into the host and protect it from nuclease degradation from the very first moment it enters the cell.\[4\]
Using single molecule experiments they showed that binding of VirE2 to ssDNA rearranges ssDNA into a compact helix even if high forces on the order of 50 pN are applied between both ends of the DNA molecule. The production of mechanical energy occurs through the free energy gain during binding of VirE2 to ssDNA. Thus, they propose that a single VirE2 protein binds to T-DNA as it enters the plant cell. This binding, occurring in a zipper-like motion, is mainly limited by thermal fluctuations of T-DNA. In a second step, the fast cooperative binding of VirE2 facilitates the formation of a helical structure and actively pulls T-DNA into the plant cytosol.\[4]\] The interaction of VirE2 molecules with each other in the absence of ssDNA underlines the cooperativity in ssDNA binding.\[64,65]\] Imaging surface plasmon resonance experiments indicate that ssDNA might coil itself onto pre-existing VirE2 aggregates due to its flexibility.\[64,66]\] This putative mechanism would further enhance the efficiency of ratcheting by VirE2. Taken together, these findings strongly suggest a translocation ratchet mechanism based on binding of VirE2 to T-DNA within the host cytoplasm.

Currently, the function of VirE2 as a chaperone in a translocation ratchet is not fully understood, because it likely has multiple functions and complex interactions with other components of the secretion system. To maintain VirE2's capability of being secreted into the host cell, the chaperone VirE1 likely prevents self-aggregation in the bacterial cytoplasm.\[67,68]\] Further, VirE2-DNA reveals an altered binding mode in the presence of VirE1.\[64] This might be important for the maintenance of the chemical potential for efficient ratcheting. In order to enter the plant cell, VirE2 is excreted by the A. tumefaciens T4SS.\[36]\] Upon contact, VirE2 is thought to associate with the cytoplasmic membrane and to form pores that
are likely to permeabilize the membrane to T-DNA. The high abundance of VirE2 within the host cytoplasm raises the question how ample amounts of it are transported into the host cytosol. A recent study reports that clathrin-mediated endocytosis is responsible for efficient uptake of VirE2 by plant cells. Apart from its role as a chaperone in T-DNA import, VirE2 serves more sophisticated functions connected to T-DNA transformation that take place after DNA translocation into the cytoplasm. Its sequence contains nuclear localization signals (NLS) that facilitate the nuclear import of VirE2 and potentially also the bound T-DNA. Interaction of VirE2 with the transcription factor VIF1 may further facilitate nuclear targeting. A recent study reports that VirE2 is trafficked actively toward the plant nucleus via the ER/F-actin network and that transport is powered by myosin XI-K.

4. Protein Secretion and the Type I Secretion System

Type I secretion systems (T1SS) mediate secretion of proteins from the cytoplasm to the extracellular environment. The secreted proteins are diverse in size and function and are often associated with acquisition of nutrients or virulence. In comparison to T2SS and T4SS, the architecture of the T1SS is relatively simple. It consists of three components: (i) The ATP binding cassette (ABC) transporter is a polytopic inner membrane protein, energizing transport by ATP hydrolysis; (ii) The membrane fusion protein (MFP) is an inner membrane protein extending into the periplasm; (iii) A TolC family protein forms a homotrimeric outer membrane pore (OMP).

4.1. Initiation of Protein Secretion by the ABC Transporter of the T1SS

At the cytoplasmic side, a non-cleavable, N-terminal signal sequence of the cargo protein interacts with the nucleotide binding domain (NBD) of the ABC transporter. This interaction triggers the assembly of the T1SS complex. Upon assembly the MFP connects the exit of the ABC transporter with the OMP opening and yields a “channel-tunnel” arrangement spanning the entire cell envelope. The C-terminus of the cargo protein inserts into the conduit and initial transport is energized by ATP hydrolysis.
power strokes triggered upon ATP hydrolysis of the ABC transporter NBD.\cite{78,80,81} The early stages of translocation are additionally facilitated by the proton motive force (pmf) across the IM.\cite{82} Only the early steps of substrate translocation depend on the ABC transporter.\cite{78,81} Once the C-terminus of the cargo protein reaches the extracellular space, Ca\(^{2+}\)-dependent protein folding can initiate the intramolecular ratchet\cite{5} (see next paragraph), suggesting that the process of “threading” depends on ATP

Table 1. Translocation processes for macromolecules for which Brownian ratchets have been discussed

| Function                      | Type of ratchet                        | Experimental evidence for ratchet mechanism |
|-------------------------------|----------------------------------------|--------------------------------------------|
| T4PS  DNA uptake during       | Chaperone-assisted \                 | Single-molecule experiments show that chaperone ComE accelerates DNA uptake; comparison of force-dependent translocation kinetics between experiment and theory provides strong evidence for translocation ratchet mechanism.\cite{3,33} |
| transformation               | translocation ratchet                 |                                            |
| T4SS  Ejection of DNA into    | Chaperone-assisted \                 | Single-molecule experiments show that cooperative binding of putative VirE2 chaperone generates force by compacting DNA.\cite{4,64} Direct evidence for translocation ratchet is missing. |
| eukaryotic target cells       | translocation ratchet                 |                                            |
| T1SS  Protein secretion       | Intramolecular \                     | Ca\(^{2+}\)-induced folding of RTX repeat segments accelerate secretion of the leucotoxin CyaA; artificial chaperones substitutes for Ca\(^{2+}\).\cite{5} Generality for other substrates is still under debate.\cite{95} |
|                               | translocation ratchet                 |                                            |
| Chaperone – usher pathway     | Biogenesis of type I and P pilis      | Brownian ratchet mechanism was proposed for directional export of pilus either through conformational changes of the pilus locking the pilus in the out position or through a plug domain that prevents pilus backtracking;\cite{97} direct experimental evidence is missing. |
| Anthrax toxin translocase     | Secretion of anthrax toxin            | Charges of the substrate and translocating channel determine translocation speed;\cite{100} structure of the translocation channel is in agreement with electrostatic ratchet model.\cite{98} |
| Sec system                    | Protein secretion \                  | Molecular dynamics simuations together with single molecule experiments suggest a new Brownian ratchet mechanism whereby ATP binding and hydrolysis bias the direction of polypeptide diffusion.\cite{100} |
|                               | Brownian ratchet                      |                                            |

T4PS, type IV pilus system; T1SS, type I secretion system; T2SS, type II secretion system; T4SS, type IV secretion system.
hydrolysis and pmf whereas the later stages of translocation are powered by a translocation ratchet.

4.2. Potentially Length-Dependent Intramolecular Translocation Ratchet for Protein Secretion

Apart from chaperone binding to rectify diffusion, translocation ratchets can also employ other chemical potentials (Figure 1). One possible source of such a potential is the concentration difference of ions between original (cis) and target (trans) compartment. Recent structural and functional data by Bumba et al. strongly support a translocation ratchet mechanism during the secretion of the Bortedella pertussis RTX leukotoxin CyaA by type I secretion systems. A non-cleavable C-terminal signal sequence mediates the initiation of transport by inserting the C-terminus in the T1SS duct. The remainder of the RTX domain consists of tandem repeats of Ca2+-binding, negatively charged non-apeptides. The RTX repeats are organized in blocks that fold into Ca2+-loaded structures termed parallel β-rolls. Accordingly, Ca2+ ions are required for folding and hence, cytotoxicity of the T1SS secreted proteins. Recent studies provide evidence for an intramolecular translocation ratchet powered by folding of the RTX-domain: Within the “channel-tunnel” system of the T1SS that spans the entire cell envelope, the secreted proteins are transported in an unfolded state, as expected from the channel dimensions (Figure 4B). The unfolded protein is transported in a C to N direction, leading to initial exposure of the C-terminus and thus the first RTX repeat segment. In contrast to the Ca2+-depleted prokaryotic cytoplasm, the Ca2+ concentration of extracellular space allows for the folding of the capping structure in front of the first RTX repeat segment. The C-terminal capping structure provides the required entropic stabilization for Ca2+-dependent folding of the first RTX repeat segment, preventing its backwards diffusion into the cytosol. The folding of the C-terminal RTX segment then triggers a translocation ratchet mechanism via segment-by-segment folding of the entire RTX domain. This cooperativity results in an acceleration of the ratcheting process that allows for translocation of the N-terminal effector domain. Interestingly, the application of external antibodies against RTX could substitute external Ca2+ with regard to functional secretion by the T1SS, thereby creating an artificial chaperone-assisted translocation ratchet that is similar to the examples above discussing DNA translocation.

Recent functional studies evoe discussion concerning the generality of the translocation ratchet mechanism in the context of the T1SS. Experiments characterizing secretion rates of hemolysin A (HlyA) by the T1SS of Escherichia coli showed that the rates are independent of the extracellular Ca2+ concentration. On the other hand, Ca2+ triggered folding of RTX repeat β-rolls accelerated CyaA substrate translocation. One possible explanation for these apparently contradictory results is given by the length of the substrates and the numbers of Ca2+-binding nonapeptides. While HlyA (1024 amino acids) harbors 6 repeats, CyaA (1706 amino acids) harbors 17 repeats. In general and in agreement with the acceleration hypothesis, proteins carrying larger N-terminal effector domains also carry a higher number of C-terminal RTX repeats suggesting that the intramolecular ratchet is most relevant for large translocating proteins.

5. Brownian Ratchet Mechanisms Are Likely to Bias Macromolecular Motion in Other Transport Systems

The mechanism of Brownian ratchets has been discussed for various other translocation systems (Table 1). For some of these systems, translocation ratchet mechanisms (that do not require ATP) have been proposed, but the experimental evidence is less well established compared to the three systems we discussed above. For other systems different Brownian ratchet models (e.g. ATP consuming, electrostatic ratchet) have been proposed. For example, for the chaperone-usher pathway a Brownian ratchet mechanism is likely to be involved in biasing pilus movement toward the extracellular side. As potential biasing mechanisms, both locking the pilus in the “out” position through conformational changes as well as a plug domain preventing pilus backtracking are discussed. A charge-state-dependent Brownian ratchet model has been proposed for secretion of anthrax toxin by Bacillus anthracis. Moreover, there is evidence that the bacterial Sec machinery employs and ATP-dependent Brownian ratchet mechanism for protein secretion. Beyond bacterial secretion, the mechanism of translocation ratchet may be involved in import of bacteriophage DNA during the infection process. In eukaryotic cells, precursor proteins are transported from the cytoplasm into mitochondria or the endoplasmatic reticulum. The translocation ratchet mechanism is one of the prevalent models for driving mechanism of protein import.

6. Conclusion

We discussed convergence of physical principles by different molecular machineries. Recent biophysical, structural, functional, and cell-biological data support the idea that Brownian motion biased through different chemical potentials on the cis and trans sides are important for DNA and protein translocation driven by different secretion systems. Thus transport can proceed even in the absence of ATP or proton motive force. A striking difference between translocation ratchets and other mechanisms of active cellular transport including cyclic, ATP-consuming molecular motors, is the low level of complexity that is required. In principle, a one-component system, such as a freely soluble, small chaperone is sufficient to bias diffusion. Thus, the genetic cost of harboring an active transport system can be kept at a minimum.

For those systems where the mechanism of translocation is discussed controversially, biophysical experiments bear strong potential to assess whether or not the translocation ratchet mechanism is correct. For example, the method explained in Box 2 may be applicable for studying protein import in mitochondria or DNA import into VirE2-expressing plant cells. For other secretion systems, combining (partial) in vitro
reconstitution of secretion systems into artificial membranes with biophysical tools seems to be a promising approach. Furthermore, a bottom-up approach generating synthetic translocation ratchets (e.g. by substituting biases in chemical potentials) would be interesting to better understand force generation by these machines.

Abbreviations

DUS, DNA uptake sequence; IM©, inner membrane (complex); OM©, outer membrane (complex); T4PS, type IV pilus system; T1SS, type I secretion system; T2SS, type II secretion system; T4SS, type IV secretion system.

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Conflict of Interest

The authors have declared no conflict of interest.

Keywords

Brownian ratchet, chemical potential, molecular motor, protein secretion, secretion system, transformation, translocation ratchet

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