Directed and persistent movement arises from mechanochemistry of the ParA/ParB system

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Edited by James A. Spudich, Stanford University School of Medicine, Stanford, CA, and approved November 10, 2015 (received for review March 13, 2015)

The segregation of DNA before cell division is essential for faithful genetic inheritance. In many bacteria, segregation of low-copy number plasmids involves an active partition system composed of a nonspecific DNA-binding ATPase, ParA, and its stimulator protein ParB. The ParA/ParB system drives directed and persistent movement of DNA cargo both in vivo and in vitro. Filament-based models akin to actin/microtubule-driven motility were proposed for plasmid segregation mediated by ParA. Recent experiments challenge this view and suggest that ParA/ParB system motility is driven by a diffusion ratchet mechanism in which ParB-coated plasmid both creates and follows a ParA gradient on the nucleoid surface. However, the detailed mechanism of ParA/ParB-mediated directed and persistent movement remains unknown. Here, we develop a theoretical model describing ParA/ParB-mediated motility. We show that the ParA/ParB system can work as a Brownian ratchet, which effectively couples the ATPase-dependent cycling of ParA–nucleoid affinity to the motion of the ParB-bound cargo. Paradoxically, this resulting processive motion relies on quenching diffusive plasmid motion through a large number of transient ParA/ParB-mediated tethers to the nucleoid surface. Our work thus sheds light on an emergent phenomenon in which nonmotor proteins work collectively via mechanochemical coupling to propel cargos—an ingenious solution shaped by evolution to cope with the lack of processive motor proteins in bacteria.

ParA ATPase | Brownian ratchet | theoretical model | motility

Fidelity of chromosome segregation is critical for cell proliferation and survival. Unlike eukaryotic mitosis, DNA segregation in bacteria is not well understood (1–3). Diffusion-based random partitioning is sufficient for the stable inheritance of small high-copy number plasmids (4). However, to ensure faithful partitioning of low-copy number plasmids of large size, active processes need to work against diffusion (or lack of it) and orchestrate directed transport and positioning. This active process is mediated by conserved tripartite segregation machineries, whose central component is a nucleoside triphosphatase (NTPase) driving the partitioning reaction. The NTPases have been classified as actin-like (ParM), tubulin-like (TubZ), or a deviant Walker-type ATPase (ParA) (5). It is clear that systems using actin or tubulin homologs function by means of filament-based pushing or pulling mechanisms (6, 7). Most low-copy number plasmids and chromosomes are, however, segregated by the ParA ATPase family (5, 8).

A series of protein–protein and protein–DNA interactions are required for ParA-mediated DNA segregation (9): the centromere region of the plasmid, marked by *parS*, recruits a large number of ParB to form a partition complex, which in turn interacts with ParA that is nonspecifically bound to the nucleoid—the large compact structure that mainly consists of the bacterial chromosome and DNA-associated RNA and proteins. Plasmid-bound ParB binds ParA and stimulates its ATPase activity, which triggers ParA dissociation from the nucleoid. Once dissociated, ParA undergoes a time delay before becoming competent again for nucleoid binding, rendering this dissociation step effectively irreversible on relevant timescales. It is unclear, however, how the ParA family of

ATPases mechanically harnesses these biochemical reactions to direct persistent movement of plasmids and ensure segregation fidelity of low-copy number genomic units.

In part based on in vitro observations that ParA tends to form fibrous aggregates at high concentrations, it has been proposed that ParAs form polymers that move plasmids by repeated polymerization/depolymerization cycles, similar to ParM or TubZ (10–13). However, recent experiments cast doubt on the feasibility of this proposal (14–17). A diffusion ratchet model was then put forward, which posited that a concentration gradient of ParA dimers on the nucleoid could act as the driving force for DNA segregation (18). This proposed mechanism gained direct support from a recent in vitro experiment that reconstituted the segregation system of the *Escherichia coli* F plasmid (14). In this experiment, the ParB protein (F SopB) specifically bound to centromere sites (F *sopC*) that were immobilized on a microbead. Upon contacting the ParA-ATP (F SopA-ATP), which was nonspecifically bound to a DNA carpet that mimicked the nucleoid surface, the microbead was observed to undergo directed and persistent movement over microns at a speed of ~0.1 μm/s. Interestingly, the microbead movement left a ParA-depleted zone in its wake, consistent with the proposed diffusion ratchet model for cargo motion. Simulations of a continuum model indicate that it is possible to drive persistent ParB-bound cargo motion by maintaining a ParA concentration gradient around the cargo if the cargo diffusion constant is reduced to that observed during persistent bead motion, which is much smaller than that of the free microbead (14, 19). However, the previous study did not address the origin of the cargo diffusion

Significance

Cells typically use processive motor proteins or the growth/shrinkage of cytoskeletal filaments to power directed and persistent movement of cellular structures. What if there are no motor proteins or filaments? Here, we establish a third mechanism of processive motility exemplified by the ParA/ParB system, which faithfully segregates low-copy number plasmids during bacterial cell division. The DNA cargos recruit ParB, which binds to and stimulates the ATPase activity of ParA bound to the nucleoid. ATP hydrolysis dissociates ParA from the nucleoid. The transient tethering arising from the ParA–ParB bonds collectively drives forward movement of the cargo and quenches lateral diffusive motions, producing a strikingly persistent trajectory. This operational principle could be important in early evolution and conserved for many systems.

Author contributions: L.H., A.G.V., K.M., K.C.N., and J.L. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1505147112/-DCSupplemental.

suppression or its potential relationship with the persistence of the microbead motion.

Whereas the spatial ParA gradient was correlated with directed and persistent movement in the *in vitro* experiments (14), key questions remained: What is the causal relationship between the motion and the gradient? What is the molecular basis for the highly persistent movement? What restricts random movements orthogonal to the principal ParA spatial gradient, where nothing but diffusion is at play according to the simple diffusion ratchet model? Finally, what is the capacity of this segregation machinery to drive directed and persistent movement of plasmids?

To address these questions, we theoretically examined the underlying principle of ParA/ParB-mediated motility. We established a theoretical model based on the setup of the *in vitro* reconstituted microbead experiment with implications for plasmid segregation *in vivo*. Through stochastic simulations, we found that bead diffusion spontaneously broke symmetry and initiated movement. The subsequent movement caused dissociation of ParA–ParB bonds at the back of the bead, whereas new bonds were established at the front. Due to the slow rate of dissociated ParA resetting its DNA-binding capability, a ParA-depleted zone emerges behind the moving bead, thus rectifying the initial asymmetry. Furthermore, the model suggests that the tethers mediated by ParA–ParB bonds not only drive directed movement but also quench the diffusive motion orthogonal to the principal direction of movement. Whereas our model is a Brownian-ratchet mechanism, it highlights the importance of the mechanochemical coupling for ParA/ParB-mediated motility. In a general sense, our model distills fundamental principles of how chemical reactions can collectively mediate directed transport without conventional motor proteins or filament-based pulling and pushing forces.

Model Development

Mechanical Model. Based on the measured diffusion constant of free microbeads near the substrate surface ($D \sim 0.1 \mu\text{m}^2/\text{s}$) and the measured microbead speed ($0.1 \mu\text{m/s}$) (14), the net driving force was estimated to be $\sim 4 \text{ fN}$ from the Einstein relation between diffusion and the viscous drag coefficient (γ): $D\gamma = k_B T$. First, let us put aside the origin of this driving force and ask the question: Can a simple mechanical model (Eq. 1), in which the microbead is propelled by this constant driving force in a fixed direction, recapitulate the observed directed and persistent movement in the presence of diffusion?

For a microscopic particle moving in a viscous medium, its Reynolds number is small, so inertial effects can be ignored. The particle motion can thus be described with the overdamped Langevin equation:

$$\gamma \frac{d\mathbf{r}(t)}{dt} = \mathbf{f}(t) + \boldsymbol{\eta}(t), \quad [1]$$

where $\mathbf{r}(t)$ is the microbead position at time t , $\mathbf{f}(t)$ is the external force acting on the bead, and $\boldsymbol{\eta}(t)$ represents random force resulting from thermal motion of the solvent molecules: $\langle \eta_i(t) \rangle = 0$ and $\langle \eta_i(t) \eta_j(t') \rangle = 2\gamma k_B T \delta_{ij} \delta(t' - t)$, where δ_{ij} is Kronecker delta and $\delta(t' - t)$ is the Dirac delta function.

The following equation was used to advance the microbead position $\mathbf{r}(t)$ over time (20):

$$\mathbf{r}(t + \Delta t) = \mathbf{r}(t) + \frac{D\mathbf{f}}{k_B T} \Delta t + \sqrt{2D\Delta t} \boldsymbol{\xi}, \quad [2]$$

where the $\boldsymbol{\xi}$ term represents a random displacement at time t , and each component of $\boldsymbol{\xi}$ satisfies a Gaussian distribution with average value zero and unit variance.

We systematically characterized the microbead movement by two parameters: speed and persistence. We adopted a definition of persistence based on that commonly used in polymer physics;

persistence is defined as the ratio between the end-to-end distance and the contour length of the movement trajectory based on the experimental bin time of 2 s. This bin time is fixed throughout the paper if not otherwise mentioned. With this definition, the maximum persistence is 1, reflecting a straight line. The persistence of the observed directed movement is >0.6 (14). With our simple mechanical model, a driving force of 4 fN could recapitulate the observed speed $\sim 0.1 \mu\text{m/s}$ (Fig. S1A). However, the microbead diffused freely in the orthogonal directions of the driving force, and the resulting persistence was low (~ 0.2) (Fig. S1A and B). Whereas the persistence increased with larger driving forces, the speed also increased (Fig. S1A). To obtain the observed movement persistence >0.6 (14), the microbead speed needs to be $\sim 0.5 \mu\text{m/s}$ (Fig. S1A), approximately five times faster than that observed (14). These significant discrepancies suggest that a simple mechanical model with a fixed driving force and intrinsic diffusion cannot explain the observed directed and persistent movement. This insight is also consistent with the conclusion from recent simulations based on a simple “chemophoresis” model (19, 21).

Mechanochemical Model. Because a constant driving force by itself cannot account for the directed and persistent movement, additional forces are needed to restrict the diffusive motion in the orthogonal directions. To address this missing component, we developed a mechanochemical model that extends the previously proposed diffusion ratchet mechanism (14). In our model, the chemical bond between ParA and ParB not only drives movement but also provides the transient tethers that quench the orthogonal excursions from the principal direction of motion. Instead of driving a power stroke as in conventional linear stepping motor proteins, ATP hydrolysis renders ParA–ParB bond dissociation events essentially irreversible, resulting in the generation and perpetuation of a spatial asymmetry in the ParA distribution around the moving cargo (i.e., a ParA concentration gradient). Thermal motion is rectified via this spatial asymmetry in binding affinity to drive directed and persistent movement. Our model essence is thus a Brownian ratchet, similar to a burnt-bridge mechanism (22, 23). However, unlike typical burnt-bridge systems in which the cargo interacts with and inactivates a single substrate site (the “bridge”) at a time, our model involves a large number of ParB bound to the cargo, interacting in parallel with a large number of surface-bound ParA. The movement reflects the collective behavior of the formation and dissociation of these bonds, which could differ substantially from conventional burnt-bridge models. For example, the driving force from individual ParA–ParB bonds does not necessarily need to overcome thermal noise and contribute directly to the persistent forward motion of a cargo, as long as the ensemble collectively produces persistent forward motion. We now set out to test the feasibility of this hypothesis.

To discern the fundamental principle, we considered the simplest scheme (Fig. 1). ParA-ATP and ParB are tethered through DNA to the substrate and microbead surface, respectively. For the initial exercise, we assumed that ParA-ATP converts to the ADP-state obligatorily and only upon binding a ParB dimer on the microbead (Fig. 1A). Because ParA-ADP rapidly dissociates from DNA, and there is a sufficiently long time delay before ParA-ADP converts back to ParA-ATP (17), we assumed that, once the ParA-ATP–ParB bond breaks, the site occupied by the ParA remains empty and incapable of binding ParA or ParB. Without explicit representation of ATP hydrolysis, we thus consolidated all reaction pathways pertaining to ATP hydrolysis into one irreversible step. Additionally, the ParA-ATP–ParB bond in the model effectively refers to the chemical bond between ParB, ParA-ATP, in addition to the DNA segments that connect the proteins to the microbead and the substrate (14, 17). From a mechanical viewpoint, this ParA-ATP–ParB bond acts as an elastic

Fig. 1. Schematic description of mechanochemical model. (A) Simplified ParA and ParB reaction scheme. (B) Top view of the model layout. (C) Side view of a series of microbead movements arising from the coupling between elasticity, formation, and dissociation of ParA-ATP-ParB bonds. For clarity, only a handful ParA and ParB dimers are shown, whereas there are hundreds to thousands of them in the simulations. The microbead surface is grafted with DNA that binds ParB dimers (green filled circles). The substrate is coated with DNA to which ParA-ATP dimers are bound (magenta ovals). The ParA-ATP-ParB bond in the model effectively refers to the DNA-ParA-ATP-ParB-DNA linkage, which acts as elastic spring and generates a restoring force when stretched. Taking into account experimental conditions, the model treats the DNA in the bond as an effective linear spring that reflects the collective behavior of the DNA on the surfaces, instead of a single polymer. This is due to the dense DNA packing on the surface and the ParB-mediated DNA bridging of closely packed DNA on the bead (*Supporting Information*). (D) Dependence of ParA-ATP-ParB bond dynamics on the bond length. (Upper) Definitions of different lengths in the model. L_e is the equilibrium length of ParA-ATP-ParB bond. L is the instantaneous ParA-ATP-ParB bond length. L_a is the maximum length of a newly formed ParA-ATP-ParB bond. X_c is the maximum bond length extension. (Lower) Dependence of ParA-ATP-ParB bond dissociation rate on bond length extension. When it extends beyond $(L_e + X_c)$, the ParA-ATP-ParB bond breaks instantaneously. Importantly, ParA-ATP and ParB can form a bond with an extended bond length, longer than the equilibrium length L_e (Fig. 1D). As the maximum energy penalty from this extension $\frac{1}{2} k_s (L_a - L_e)^2$ is less than $k_B T$, newly formed bonds are typically prestretched by thermal energy. When these prestretched bonds drive the microbead to move rightward (as in Fig. 1B), the bonds near the left end of the bead dissociate. The bond dissociation is assumed to immediately convert the ParA-ATP to ParA-ADP (blue rectangles) that releases from the substrate without delay. The resulting vacancy thus remains noninteracting throughout the simulation (gray rectangle). As the bead forms more new prestretched bonds to its right than to its left, the asymmetry in the bonds drives further rightward movement. This way, the irreversible ParA-ATP-ParB bond dissociation rectifies the thermally driven movement, perpetuating the directional movement. Hence, the model essence is a Brownian ratchet that harnesses the mechanochemical feedback between chemical bond dynamics, mechanical force, and bead movement.

spring (Fig. 1B and C), which implicitly includes DNA elasticity influenced by DNA crowding and ParB-mediated DNA cross-linking (*Supporting Information*). The deformation of this spring could generate restoring forces on the bead. Collectively across the bead surface, the vector sum of many ParA-ATP-ParB bonds could thus generate a net driving force that displaces the bead. Whereas the ParA-ATP-ParB bond has an intrinsic dissociation rate, the bond length change (hence the tensile force change) associated with the bead movement alters the ParA-ATP-ParB bond dissociation kinetics. Like a typical chemical bond, the ParA-ATP-ParB bond is weakened by force and breaks if stretched beyond a critical limit by the bead movement. This dissociation results in the depletion of ParA behind the bead. Meanwhile, ParB on the leading edge continues to establish new bonds with ParA on the unexplored regions of the substrate, where the ParA-ATP concentration is higher. The microbead movement therefore maintains the asymmetric biochemical environment that in turn supports further forward movement. Thus,

the essential feature of this model hinges on the nature of the mechanochemical coupling.

We quantitatively studied the dynamics of microbead movement by Brownian dynamics simulations. Because the bead does not roll during persistent movement (14), only its proximal portion contacting the substrate is relevant. The model therefore simplified the microbead as a flat circular disk. In simulations, ParA-ATP was initially distributed with 10-nm spacing uniformly over the substrate surface. A total of 5,000 ParB dimers was uniformly distributed over the surface of the disk that has a diameter of 1 μm . The positions of ParA and ParB in the model represent the anchoring points of the DNA molecules on the substrate and the cargo disk surface, respectively, to which the proteins bind. We modeled the ParA-ATP-ParB bond as an elastic spring. The vertical distance between the substrate and the disk was fixed at the assumed equilibrium bond length of DNA-ParA-ATP-ParB-DNA (L_e) throughout the simulation (Fig. 1D). The subsequent stochastic reactions between ParA

and ParB were simulated with the kinetic Monte Carlo scheme. At each simulation time step, each ParB dimer could interact with the available ParA-ATP within a distance L_a (Fig. 1D), and bind only one ParA at a time. The probability of binding is proportional to $\exp(-\frac{1}{2}k_s(L-L_c)^2/k_BT)$ for $L_a \geq L \geq L_c$; otherwise, it is zero. Here, k_s is the spring constant of the bond. L denotes the separation between the ParB and the ParA-ATP. If this bond forms, L is the instantaneous bond length, and $\frac{1}{2}k_s(L-L_c)^2$ represents the associated elastic energy penalty. Importantly, given the model parameters (Table S1), the maximum of this energy penalty is less than thermal energy k_BT . Consequently, thermal energy can be readily harnessed to pre-stretch the new bond, which in turn provides an elastic force. In the simulation, we summed the elastic forces from all of the ParA-ATP-ParB bonds over the disk surface. This net force coupled with diffusion drives the motion of the microbead for one time step. The model ignores the force in the z direction, which is balanced by the combination of magnetic force, gravitation, and charge repulsion between the bead and substrate in our in vitro experiments. In the next time step, the bond lengths of the ParA-ATP-ParB complexes were updated by this motion, from which the dissociation rates of the existing ParA-ATP-ParB complexes were calculated according to the following force dependence (Fig. 1D): When the bond extension $(L-L_c) > X_c$, the bond breaks instantaneously; otherwise, the dissociation rate is determined from $k_{\text{off}}(f) = k_{\text{off}}^0 e^{f/f_c}$, where k_{off}^0 is the intrinsic bond dissociation rate, f is the elastic force from the bond stretching $f = k_s(L-L_c)$, and f_c is the characteristic bond dissociation force. The dissociation reaction was next implemented in the stochastic simulation. Meanwhile, because of the bead movement in the previous time step, ParBs that were not in the ParA-bound form could now explore the new territory and form bonds with available ParAs. We then updated the net force from all of the ParA-ATP-ParB bonds, including changes in the existing and the newly formed complexes. The bead movement was then calculated as in the previous time step. We repeated these steps throughout the simulation over time.

We stress that the model essence remains robust over a broad range of model parameter (Fig. S2) and does not depend on the specific force dependence of the dissociation rate, as other force-dependent bond dissociation models work equally well (Fig. S2). The model parameters were estimated from existing experimental measurements wherever possible (see Table S1 and the parameter derivations in Supporting Information, *Model Parameter Consideration or Estimation*). Unless otherwise noted, the Brownian dynamics time step Δt was set to 10 μ s in the simulations, which is small enough to account for the fastest reaction/diffusion process in the system. Choosing smaller time steps did not affect the model results (Fig. S3). Below, we first present typical model results.

Results

Directed and Persistent Movement Emerges from a Mechanochemical Model of ParA-ParB Interactions. Fig. 2A shows an example of directed and persistent movement of a microbead generated by our mechanochemical model. According to the model, stochastic fluctuations triggered the initial symmetry-breaking event. Snapshots of the chemical bond distributions on the microbead revealed drastic changes during symmetry breaking (Fig. 2B). At the beginning, the nonuniform distribution of forces from the stochastically formed chemical bonds together with the diffusive motion of the microbead drove its initial movement, which subsequently induced the dissociation of bonds at the trailing end. Bond dissociation is coupled to the formation of ParA-ADP, which dissociates from the substrate. As the bead moved forward, it established new ParA-ATP-ParB bonds in the new territory, which provided the net force that drove the bead forward. Consequently, the initial asymmetry was perpetuated and

the ensuing bead movement was directed and persistent. There was no preference in the direction of persistent movement (Fig. S4), in line with experimental observations (14).

Furthermore, the model predicted the differential spatial pattern of ParA around the microbead (Fig. 2A and B). Whereas the leading edge was enriched in ParA-ATP-ParB bonds, the microbead left a path of ParA depletion in its wake. The microbead movement essentially burnt its own bridge and there was no way back. This self-generated polarity provided an overall directed driving force that was in the persistent direction. Note that, if we incorporated the diffusion of ParA molecules, ParA-ATP re-binding to substrate, and an explicit reverse reaction in the initial binding step, the sharp boundary of the ParA depletion zone behind the microbead was smoothed out (Fig. S5), remarkably resembling experimental observations (14). Importantly, including these additional factors did not qualitatively affect the directed and persistent movement (Fig. S5). We therefore ignored these more detailed processes for the remainder of the results, keeping the model minimal.

The above results are consistent with the proposed diffusion ratchet mechanism (14, 18). However, the important question left unanswered by the previous proposal is: What restricts microbead deviation orthogonal to the principal movement direction? The answer lies in the tethering of the microbead to the substrate by the ParA-ATP-ParB bonds. Fig. 2C shows the temporal evolution of the bond number during microbead movement. At steady state, there were ~ 250 bonds for this set of model parameters, analogous to 250 springs tethering the microbead to the substrate. From the viewpoint of the energy landscape, these tethers imposed an energy barrier quenching diffusion (Fig. 2D). To calculate the tethering effects on the motion, we chose a time point at which the microbead was undergoing steady-state movement, and we virtually moved it through various displacements without changing the status of existing bonds. We then calculated the resulting changes in the elastic energy from these chemical bonds. As shown in Fig. 2D, an orthogonal deviation of 5 nm incurred an energy penalty of $\sim 2.5 k_BT$. These bonds collectively defined a potential that trapped the microbead and suppressed lateral deviations. Importantly, the force involved at each time step was in the piconewton range as indicated by the energy landscape (energy/displacement) (Fig. 2D). However, because the microbead movement driven by chemical bond-mediated forces was gated by the dissociation of these bonds, this effective internal resistance greatly slowed the speed. Consequently, the net driving force in the femtonewton range equaled the difference between the instantaneous driving force and the chemical bond-mediated internal resistance. That is, these chemical bonds not only provided the driving force for microbead movement but also suppressed deviations in the orthogonal direction and slowed directed motion. This finding resolves a point of confusion concerning plasmid diffusion constants in vivo. The measured apparent diffusion constant of plasmid movement during directed movement in vivo is small (24), which has been interpreted as an indication that diffusion ratchet mechanisms are not capable of generating directed plasmid movement (24). Our results demonstrate that the slow apparent diffusion is a natural consequence of free diffusion of the plasmid constrained by the ParA-ATP-ParB bonds, similar to our microbead experiments (14). Our model thus predicts that, instead of diffusion-limited motion, the mechanochemical coupling ensures directed and persistent movement.

The Number of ParA-ATP-ParB Bonds and Cargo Size Influence Movement Persistence and Velocity. We next investigated how the interplay between the number of ParA-ATP-ParB bonds and the intrinsic mobility of the cargo influence cargo movement. Multiple contributing factors determine the bond number, e.g., the on and off rates, the densities of ParA and ParB on the substrate and on the

Fig. 2. Persistent movement of microbead arises from the mechanochemical model. (A) A typical trajectory (gray path) of directed and persistent movement of a microbead. (B) The top views of the model simulation snapshots showing the spatial-temporal evolution of ParA binding states during the directed and persistent microbead movement as in A. Here, ParA-ATP that does not bind to ParB is in magenta, the ParA depletion zone is in gray, and the ParA-ATP that forms ParA-ATP-ParB bond is in blue. The green dashed line marks the boundary of the microbead. For clarity, only 25% of the ParA-ATP-ParB bonds are shown in the figure. (C) Time evolution of the ParA-ATP-ParB bond number as in A. (D) A typical energy landscape of the microbead movement on the substrate. Here, we plotted the energy penalty incurred by virtual displacements (δx and δy) from the instantaneous position of the microbead (marked by the pink circular disk). These virtual displacements do not involve bond formation or dissociation. The green star marks the lowest energy displacement, which has a negative energy barrier. That is, there exists a driving force propelling the microbead toward the location of this lowest energy point, consistent with the overall persistent direction of movement (marked by the arrow).

microbead, respectively. We focused on the effect of ParB density and bead size on the directed and persistent movement.

The model predicted that, for a given bead size and hence a fixed intrinsic diffusion constant, there existed a threshold ParB density on the bead below which persistent movement could not be maintained (Fig. 3A). For a cargo of 0.5- μm radius (diffusion constant of $\sim 0.1 \mu\text{m}^2/\text{s}$ as in ref. 14), the minimal density of ParB is $\sim 1,000/\mu\text{m}^2$, consistent with estimates of ParB density on P1 plasmid of approximately one dimer per 30 bp (25). This points to the critical role of the “ParB-spreading” process in which ParB binding to *parS* on the plasmid nucleates higher-order complex assembly involving a large number of ParBs to promote plasmid segregation (26–30). Conversely, if the density of ParB was too high, too many ParA-ParB bonds formed resulting in the de-

crease of both the persistence and the speed (Fig. 3A–C). Similar results hold for ParA density. Generally speaking, too many bonds effectively trapped the cargo. Our model thus provides an explanation for the frequent loss of plasmids during cell division when either ParA or ParB are in excess (31). According to our model, these phenotypes could arise from the loss of directed and persistent movement by the ParA/ParB-mediated transport machinery as plasmids become stuck to nucleoid by too many ParA-ATP-ParB bonds. Last, there appeared to be a critical cargo size below which diffusive movement persisted. These findings are consistent with observations that, although in principle the ParA/B system could mediate transport of diverse cargos, these cargos tend to be massive (32). The directed and persistent movement was favorable for beads of intermediate

Fig. 5. Saltatory movement reflects competition between ParA-ATP-ParB bond chemistry and mechanics. (A) A representative trajectory of saltatory movement from simulations. (B) The number of ParA-ATP-ParB bonds during the saltatory movement in A. (C) A representative saltatory trajectory of a microbead from in vitro experiments. (D) The instantaneous microbead step size (red line) and the instantaneous fluorescence intensity (green line) of the microbead, which due to the TIRF illumination is inversely proportional to the distance of the microbead from the substrate, during the saltatory movement in C. For A and C, points on the x-y plot correspond to the position of the center of the 1-μm microbead at 2-s intervals.

rapid jumps, after which it was again confined. During this process, the number of ParA-ATP-ParB bonds oscillated, with more bonds corresponding to confined diffusion and fewer bonds corresponding to jumps (Fig. 5A and B). The model suggested that this jerky movement occurred due to the asynchrony between the mechanical action and the chemical reaction of the ParA-ATP-ParB bonds. Whereas the mechanical forces were sufficient to drive the microbead forward, the chemical bonds persisted and kept the bead tethered. With the rupture of a sufficient number of bonds, the bead could undergo sizable movement (a jump) driven by the mechanical force. Once a significant fraction of bonds broke, the remaining bonds were insufficient to quench diffusion. Subsequent diffusion not only introduced randomness in the movement direction but also prevented tethering and drove larger strides until a sufficient number of ParA-ATP-ParB bonds were reestablished to quench diffusion. Consequently, the number of ParA-ATP-ParB bonds negatively correlated with the step size during saltatory movement (Fig. 5A and B). This saltatory movement thus manifested another facet of the underlying mechanochemical coupling mechanism and constituted a unique prediction of the model, which was absent from previous simple diffusion ratchet models (14, 19, 21).

To test this prediction, we analyzed the trajectories of microbead movements in reconstitution experiments previously described (14). The modes of microbead movement were diverse, likely reflecting heterogeneity with respect to the surface densities of SopA, SopB, and DNA, and the variation in the surface confinement force. Specifically, whereas some beads underwent directed persistent movement and some were stuck to the substrate, there was a sizable fraction of microbeads that displayed saltatory movement, alternating between confined diffusive motion and long strides (Fig. 5C). We also observed that the proximity of the SopB-coated microbead to the DNA-coated surface, as inferred from the microbead fluorescence intensity in the exponentially decaying evanescent field generated by total internal reflection, was anticorrelated with the step size of the microbead movement with average correlation coefficient of -7.1 ± 4.1 ($n = 12$).

Namely, the microbead fluorescence intensity decreased during long strides in the saltatory movement (Fig. 5D). The microbead fluorescence intensity decrease in the total internal reflection fluorescence (TIRF) field reflects an increase in the microbead-substrate distance and vice versa. We interpreted the transient “hopping” of the microbead away from the substrate as evidence for loss of these tethering bonds.

ParA/ParB-mediated plasmid motility *in vivo* has been shown to vary. “Smooth” translocation, where the plasmid continuously moves close to the receding edge of a ParA (or SopA) depletion zone on the nucleoid, has been observed *in vivo* for pB171 plasmid and F plasmid (33). More recently, both plasmids have also been shown to support saltatory movements, whereby the plasmids are for the most part immobile and uniformly distributed at relatively fixed positions on the nucleoid (34). For P1 plasmid, ParA distributes uniformly over the nucleoid and also forms colocalized foci with immobile plasmids (16). During plasmid movement, these colocalized ParA foci disappear and only reappear once the plasmids have been repositioned. These “stick-and-move” dynamics are consistent with the “saltatory” regime of the diffusion ratchet model described here. We conclude that diverse modes of ParA/ParB-mediated motility likely use the same diffusion ratchet mechanism, but subtle differences, some of which are emphasized here, could produce transitions between smooth and saltatory modes of plasmid movement observed *in vivo*.

Our combined work thus suggests that directed and persistent movement requires a sufficient number of ParA-ATP-ParB bonds to transiently tether the microbead and quench diffusion while still being able to dissociate rapidly enough to drive microbead movement. The proper coordination between the timescales of the mechanical action and the chemistry of the ParA-ATP-ParB bonds orchestrates the synchrony between bond tethering and dissociation in driving persistent cargo movement.

Response of ParA/ParB-Mediated Motility to an Opposing Force. Last, we investigated how ParA/ParB-mediated motility responds to opposing forces. Fig. 6A shows representative simulation trajectories

Fig. 6. Response of ParA/ParB-mediated directed movement to opposing force. (A) Model predictions for diversions of ParA/ParB-driven movement of microbead by an opposing force with constant direction and magnitude. Example trajectories for the cases of small ($F = 30\text{ fN}$) and large ($F = 1\text{ pN}$) opposing forces are shown. For comparison, the corresponding trajectory when there is no external force ($F = 0$) is shown (light blue line); the original direction of this trajectory is marked by the blue arrow ("V"). The gold dot on the trajectory marks when the external force is switched on in the simulation, and trajectories of the subsequent movement are shown in different colors (dark blue for $F = 30\text{ fN}$, and green for $F = 1\text{ pN}$). (B) Dependence of microbead speed on opposing force. The blue-shaded zone marks the regime in which the external force breaks the existing bonds and drives the microbead movement in a purely mechanical manner. The resulting speed of the microbead is thus independent of ParA/ParB system. (C) Experimental observation of moving microbeads diverted by repulsive force from stuck magnetic beads. Representative trajectories of five SopA/SopB driven beads (colored heavy lines) moving on the surface of the flow cell interacting with a stuck bead (red dot in middle of white circle). Due to the external magnetic field, the magnetic moments of the beads are aligned perpendicular to the plane of motion, which results in a repulsive interaction between beads that falls off as the inverse fourth power of their separation. (D) Simulated trajectories of the microbead movement in the presence of the same magnetic dipole-dipole interaction as in C. The position of the virtual microbead is indicated by the red dot, and five independent trajectories are shown. Note that C and D share the same force scale: The log of the repulsive force in the vicinity of the stuck beads is represented by the contour lines (spaced every 0.5 log units from the maximum of 1 pN at the purple contour) and the white shading (force scale on right side of Fig. 6C).

of the microbead in which a force was applied in the opposite direction of the bead movement after the bead began directed and persistent movement. At a small opposing force ($\sim 0.03\text{ pN}$), the microbead simply diverted its path (Fig. 6A). A larger force of 1 pN or more caused the microbead to turn sharply and move backward (Fig. 6A). Regardless of the magnitude of the opposing force, ParA/ParB-mediated motility never stalled. The model predicted that, whereas an opposing force could readily alter the direction of the ParA/ParB-mediated motility over time, it did not change the speed very much until the opposing force significantly exceeded 1 pN (Fig. 6B). In the latter case, the large external force broke the bonds and drove the microbead directly, which did not reflect the inherent ParA/ParB-mediated motility.

The predicted trajectory diversion and resilience of speed in the regime of directed and persistent movements are natural consequences of the diffusion ratchet mechanism. The resisting force presents a bias to the stochastic fluctuations that gradually remodel the distribution of ParA-ATP-ParB bonds over time, and ultimately drive the microbead in the direction of the force. Such behavior is in contrast to conventional motor proteins such as kinesin, whose motility and hence stepping uses the chemical energy in a more deterministic manner. In other words, the Brownian-ratchet nature of ParA/ParB-mediated motility has the capacity to avoid obstacles, which may be important for transporting large cargos inside bacteria.

To experimentally examine this prediction, we exploited an experimental configuration in which some magnetic beads stuck on the substrate (probably due to a higher than average density of

sopC-SopB on the bead), whereas others underwent directed and persistent movement (Fig. 6C). The external magnetic field aligns the magnetic moments of the beads perpendicular to the x - y plane, which results in a repulsive dipole-dipole interaction between beads that falls off as the inverse fourth power of their separation. The fixed beads therefore produced a distance-dependent opposing force against the moving beads, the magnitude of which can be determined from the known magnetic properties of the beads and the applied magnetic field (14). We observed that the microbeads moving in head-on paths toward a fixed bead got diverted more than those moving marginally (Fig. 6C). The speed changes somewhat after the diversion ($27 \pm 40\%$, $n = 6$). Our model simulation with the same distance-dependent opposing force shows the same behavior (Fig. 6D). Our experimental observation thus confirms this diversion behavior, which highlights the underlying mechanism as Brownian ratchet rather than a more deterministic mechanism.

Discussion and Conclusions

In this study, we developed a theoretical model for ParA/ParB-mediated directed and persistent cargo motility observed both in vitro and in vivo. Our model adds to the growing body of data refuting the filament-based models for ParA-mediated plasmid motion.

Combining the mechanistic insights from the model with experimental results, we arrived at the following picture of the ParA/B-mediated directed and persistent motion (also see our analytic results in [Supporting Information](#) and Fig. S6). Initially, with the depletion of the ParA-ATPs directly underneath the

microbead (Fig. 2 and Fig. S64), thermal fluctuations displace the microbead in a random direction without causing significant restoring forces, thus breaking symmetry. This displacement makes more ParA-ATP available for ParB binding on one side of the microbead, subsequently referred to as the front. Because the energy penalty associated with the bond extension is small [e.g., $\frac{1}{2} k_s (L_a - L_c)^2 < k_B T$], newly formed ParA-ATP-ParB bonds are typically prestretched by thermal fluctuations (i.e., $L_a > L_c$; Fig. 1D and Fig. S64). The asymmetric ParA-ATP distribution around the microbead thus creates conditions favoring the formation of more forward-stretched new bonds at the front than the backward-stretched bonds at the rear (Fig. S64). This results in a net forward bias of the stretched bond orientation, and further drives the microbead movement in the direction of the displacement. The resulting asymmetric chemical environment (i.e., binding site distribution) serves to rectify thermal fluctuations of the cargo and perpetuate the directed and persistent movement. This is characteristic of a Brownian ratchet.

At steady state, we derived an analytic expression for the ParA/ParB-mediated motility speed based on a mean-field approximation of the model (Supporting Information). It suggests that the speed is proportional to the maximum lateral distance between the ParB on the microbead and the ParA-ATP on the substrate for which binding can occur (i.e., $\sqrt{L_a^2 - L_c^2}$; Fig. 1D). This maximum lateral distance is analogous to the step size of a linear stepper motor protein. Additionally, the speed is inversely proportional to the effective timescale arising from the interplay between the mechanical response of the system and the chemical reactions of ParA-ATP-ParB bond formation and turnover. This effective timescale in ParA/ParB-mediated motility is thus similar to the duration of ATP hydrolysis cycles in conventional motor proteins.

However, we emphasize that our mechanism fundamentally differs from the stepping mechanism of conventional molecular motors. Although ATP hydrolysis is not explicitly described in the model, it is involved in the irreversible ParB-stimulated dissociation of ParA from the DNA carpet. Consequently, instead of directly driving a power stroke as in conventional motors, ATP hydrolysis is primarily involved in generating the ParA depletion zone behind the moving cargo. Whereas the spatial ParA concentration gradient of the ParA-depleted zone drives forward movement, it does not impose a priori constraint on lateral diffusive movement. Persistent movement requires a sufficient number of ParA-ATP-ParB bonds to tether the cargo and quench diffusive motion in the lateral directions while dissociating frequently enough to allow forward motion (Figs. 2–5). We note that this tethering effect opposes lateral excursions with a shallow energy barrier of approximately a few $k_B T$ (Fig. 2D); escaping from the potential well becomes probabilistic. Indeed, significant lateral excursions were predicted to occur occasionally (Fig. S7), consistent with the *in vitro* experiments (14). Together, the mechanochemical coupling of the ParA-ATP-ParB chemical bonds drives the forward cargo motion, imposes resistance to the forward motion, and constrains diffusive course deviation ensuring persistent movement (Figs. 2–5). The transient tethering aspect of the model provides an explanation for the apparent reduced diffusion constants measured in both *in vitro* and *in vivo* experiments (14, 24, 35).

Recently, a “DNA relay” model proposed that, for the *Caulobacter crescentus* ParABS system, DNA-bound ParA-ATP dimers tether cargo to the bacterial chromosome but require chromosomal elastic dynamics to generate the translocation force that “relays” cargo from one DNA region to another (35). This mechanical contribution from the chromosome was evoked because simulations of a simple “diffusion-binding” model indicated that the cargo diffused too slowly to produce directed cargo movement (35). However, the diffusion constant of the cargo was likely significantly underestimated in this simulation. The modeling was

performed with the measured diffusion constant (35), rather than an effective free diffusion constant chosen to produce simulation results that recapitulate the experimental data, including the measured diffusion constant. As a result, the decrease in diffusion due to tethering was effectively doubled because the experimentally determined diffusion constant, which necessarily includes the hindering effects of the tethering process, was input into the model that further reduced the effective diffusion due to tethering of the cargo in the Brownian simulations (35). Furthermore, the DNA relay model requires a ParA/ParB bond lifetime that far exceeds measured values of the bond lifetime for related Par and Sop systems (17). Refinements in modeling require further experimental delineation of mechanistic details of the apparently different behaviors displayed by different ParABS systems.

In our model, the ParA/ParB-mediated Brownian-ratchet motility is mechanically driven by the collective dynamics of an ensemble of bonds. We note that the Brownian-ratchet mechanism does not strictly depend on the direct mechanical pulling force of individual bonds, as it solely hinges on how the asymmetry of the system rectifies thermal fluctuations. The cargo could therefore undergo directed motion through a simple burnt-bridge Brownian-ratchet mechanism without chemical bond-mediated mechanical forces. However, in the absence of mechanical force, the cargo is not tethered. Consequently, movement persistency cannot be readily maintained, as nothing prevents diffusive excursions in the orthogonal directions of the directed motion, similar to the predicted case with a very small bond spring constant (Fig. 4).

The mechanochemical coupling mechanism examined here does not constrain certain aspects of the biochemical parameters, e.g., the temporal sequence of ParA-ATP-ParB bond dissociation and ATP hydrolysis. The model assumes a cutoff bond length beyond which the ParA-ATP-ParB bond dissociates immediately. The model essence is preserved when this cutoff length varies (Fig. S2C). Moreover, most of the bonds spontaneously dissociate at steady state in the model rather than being stretched to the breaking point. Similar dissociation kinetics could arise from more elaborate biochemical dissociation pathways in reality including multiple substeps or nonlinear terms. Quantitative biochemical characterization of ParB-stimulated ATP hydrolysis of DNA-bound ParA-ATP will assist further refinement of the model in the future.

Last, because bacteria do not have typical linear stepper motors, the operational principle of ParA/ParB system perhaps played important roles in a variety of biological systems since early evolution, and could thus be conserved in many systems. In fact, this driving-quenching mechanism is similar to the “Pac-Man”-type motility evidenced for the DAM1 ring (36, 37). The DAM1 ring directly connects chromosomes “end-on” to microtubules in yeast mitosis. Whereas the Dam1 ring is highly diffusive (36), its end-on binding to microtubule suppresses its diffusive excursions and allows it to steadily track the depolymerizing microtubule end, driving chromosome movement with high fidelity in mitosis (38). Our study distills a general physical principle for collective behavior from properly coordinated chemical reactions. Unlike classical molecular motors, the ParA/ParB system does not have an inherent directionality. Directionality arises from symmetry breaking and self-organization without the guide of a well-defined track. The subsequent motion diverts its direction upon encountering an opposing force, instead of stalling (Fig. 6). Such flexibility reflects the fact that ParA/ParB-mediated motility is driven by the collective behavior of many weak Brownian ratchet-type interactions. Because the cargo motion depends on the ParA depletion zone, multiple cargos in proximity mutually influence their motion through interaction of the depletion zones. Thus, the ParA-based cargo transport systems have been proposed

to be able to self-organize equidistant distribution of multiple cargos for partition in vivo. Dissecting these additional features in bacterial cell division will be our future topic of research.

Methods

Proteins. Protein expression, purification, and labeling were performed as previously described in detail (39).

DNA-Carpeted Flow Cell. Flow cell assembly, pacification, and carpeting with sonicated salmon sperm DNA was done as previously described in detail (39).

DNA-Bead Coupling. A biotinylated and Alexa 647-labeled DNA fragment (3.36 kb) containing a *sopC* centromere site was PCR amplified and coupled to MyOne Streptavidin C1 Dynal beads (Invitrogen) as previously described in detail (39).

Imaging and Analysis. The illumination, microscope, and camera settings were previously described (14).

ACKNOWLEDGMENTS. We thank the reviewers for their constructive suggestions that greatly improved the quality of the paper. L.H., K.C.N., and J.L. are supported by National Heart, Lung, and Blood Institute intramural research program at NIH. A.G.V. and K.M. are supported by National Institute of Diabetes and Digestive and Kidney Diseases intramural research program.

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