A mathematical model of ParA filament-mediated chromosome movement in Caulobacter crescentus

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HIGHLIGHTS

- We model ParA/ParB-mediated chromosome segregation in Caulobacter crescentus.
- ParB binding and diffusion with ParA depolymerization yield directed ParB movement.
- The protein TipN controls ParB movement by altering ParA cytoplasmic concentrations.
- Control of ParA polymerization is essential for proper chromosome segregation.

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ABSTRACT

Caulobacter crescentus uses the dynamic interactions between ParA and ParB proteins to segregate copies of its circular chromosome. In this paper, we develop two mathematical models of the movement of the circular chromosome of this bacterium during division. In the first model, posed as a set of stochastic differential equations (SDE), we propose that a simple biased diffusion mechanism for ParB/ParA interactions can reproduce the observed patterns of ParB and ParA localization in the cell. The second model, posed as a set of nonlinear partial differential equations, is a continuous treatment of the problem where we use results from the SDE model to describe ParB/ParA interactions and we also track ParA monomer dynamics in the cytoplasm. For both models, we show that if ParB complexes bind weakly and nonspecifically to ParA filaments, then they can closely track and move with the edge of a shrinking ParA filament bundle. Unidirectional chromosome movement occurs when ParB complexes have a passive role in depolymerizing ParA filaments. Finally, we show that tight control of ParA filament dynamics is essential for proper segregation.

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1. Introduction

Tight regulation of DNA segregation dynamics is central to the proper progression of cell division. In eukaryotic cells, the mitotic spindle's role in facilitating chromosome movement is well characterized (Mogilner and Craig, 2010). Several theoretical models for chromosome motility driven by microtubule depolymerization have been put forward in the past few years. Various theories have been explored, such as biased diffusion of chromosomes on dynamic microtubules (Hill, 1985; Joglekar and Hunt, 2002; Shtylla and Keener, 2011), coupler-driven motility using conformational changes of depolymerizing microtubules (Peskin and Oster, 1985; Molodtsov et al., 2005; Efremov et al., 2007), and fibril-based dynamic binding by kinetochore (McIntosh et al., 2008). The interested reader is referred to Vladimirov et al. (2011) for and in-depth review of chromosome motility modeling.

On the other hand, it is not well understood how bacteria segregate their DNA. Recent experiments (Toro et al., 2008; Ptacin et al., 2010), however, have provided evidence that bacterial chromosome segregation is an active process which shares some similarities with the segregation apparatus of eukaryotic cells.

Caulobacter crescentus (Caulobacter hereafter) is an important model system for the study of DNA segregation mechanics in bacteria. Caulobacter has a single densely packed circular chromosome that spans the entire length of the cell (Cabeen and Jacobs-Wagner, 2010). During chromosome replication, one chromosome copy is moved with the help of several proteins. The two key components necessary for chromosome segregation are the proteins ParA and ParB (Toro et al., 2008; Gerdes et al., 2010). Similar to actin, ParA monomers first bind ATP and then assemble into dimers that are recuit into growing ParA filaments. ParAs are deviant Walker-type ATPases with weak ATPase activity. ParBs
are thought to interact with ParAs through their N-terminal ends and also stimulate ATP hydrolysis, which causes ParA filaments to depolymerize (Easter and Gober, 2002; Leonard et al., 2005; Barilla et al., 2007). Further, ParB proteins bind origin-proximal parS DNA sequences, which leads to the assembly of centromere-like regions on the chromosome, similar to eukaryotes. When chromosome replication is initiated in these cells, two ParB complexes assemble on each copy; one ParB complex is anchored to the cell membrane and the other one is left free in the cytoplasm. Next, ParA assembles into dynamic “comet” clouds that interact with the free ParB. This interaction leads to the translocation of ParB $\approx 3 \mu$m across the cell by holding on to the edge of a depolymerizing ParA cloud, pulling apart the chromosome copies as it moves (Ptacin et al., 2010; Viollier et al., 2004; Fogel and Waldor, 2006; Schofield et al., 2010; Shebelut et al., 2010).

The mechanisms underlying this chromosome movement are not well understood. An important obstacle has to do with the lack of detail about the arrangement and orientation of ParA filaments inside the dynamic ParA clouds. Recent in vivo observations in Caulobacter, however, indicate that ParAs are assembled into linear structures (or bundles) oriented along the long axis of the cell (Ptacin et al., 2010). Similar to eukaryotic mitosis, there is evidence that chromosome segregation follows an ordered pattern of events in Caulobacter (Shebelut et al., 2010). In this paper, we focus our attention on the commitment stage of chromosome translocation where ParB engages irreversibly with ParA clouds and rapidly translocates to the new pole. Specifically, there are two important observations about chromosome motility which we seek to capture with our modeling. First, in Caulobacter, the chromosome region bound by ParB translocates to the opposite side of the cell following a unidirectional path (Ptacin et al., 2010; Schofield et al., 2010; Shebelut et al., 2010). Second, the position of moving ParBs in the cell correlates with retracting ParA cloud edges (Fogel and Waldor, 2006; Schofield et al., 2010; Shebelut et al., 2010).

Proteins that localize at the cell poles have been implicated in the control of chromosome movement of Caulobacter (Ptacin et al., 2010; Schofield et al., 2010; Lam et al., 2006). One such protein is TipN, which localizes at the new pole and has been shown to be important for unidirectional ParB movement during segregation. In $\Delta$tipN cells, ParB frequently changes direction and pauses (Ptacin et al., 2010; Schofield et al., 2010). Simultaneously, when TipN is depleted, ParB proteins appear close to the old pole, in contrast with wild type ParA localization patterns (Schofield et al., 2010). There is evidence that there are direct interactions between ParA monomers and TipN (Ptacin et al., 2010). TipN/ParA interactions are also supported by the accumulations of ParA proteins at the new pole in wild type cells (Schofield et al., 2010). It is not clear how proteins that localize at one end of the cell, such as TipN, can affect the movement of ParA proteins that are located several microns away (Ptacin et al., 2010; Schofield et al., 2010).

The movement of bacterial chromosomes during division has been previously studied with the help of computational modeling. In Jun and Mulder (2006), a model that used entropic forces as the sole agent for bacterial chromosome segregation was constructed. But, for the size and organization of the DNA of Caulobacter, entropy alone is not sufficient to drive segregation. Furthermore, for Caulobacter, it has been shown that ParA is necessary to initiate the directed movement of a replicated chromosome copy (Toro et al., 2008). Also, a stochastic computational model of Ringgaard et al. (2010) was proposed for a related ParAB segregation mechanism operating in E. coli, where a Par network works to move plasmids. ParA proteins in this bacterium are positioned in between the plasmid copies, which, in contrast to the Caulobacter chromosome copy, experience frequent switching in direction of movement as they migrate to the two cell halves. In Ringgaard et al. (2010), proper plasmid localization could only be obtained if ParB detachment rates were made to be ParA filament length-dependent. This key assumption is not well motivated.

Concurrently with this work, a computational model based on Brownian dynamics simulations was employed to study Caulobacter chromosome movement in Banigan et al. (2011). A key assumption in their simulations was that ParB organizes into several long continuous filaments that engage with individual ParA filaments. We are not aware of data that supports this assumed ParB structure. Further, in their model, the authors included a high level of detail, whereby the individual motion of every ParB and ParA protein ($\approx 2000$ proteins) was followed. In contrast, here we take a simplified approach, in which we do not follow each ParA/ParB protein individually, but instead follow only the basic ingredients necessary to generate and sustain movement. Further, here we are interested in studying the control of movement by TipN and its interactions with cytoplasmic ParA monomers and dimers; these issues were not examined by Banigan et al. (2011).

In this paper, we construct mathematical models, based on the new biological data available, which describe the dynamics of the ParAB machinery in Caulobacter. Our primary assumption is that ParA clouds are made up of continuous ParA filament bundles that remain anchored at a cell end during division. We test various ParB/ParA interaction scenarios and compare the resulting ParB movement and ParA localization with the available experimental data. Specifically, in this paper we construct two mathematical models for the underlying ParA/chromosome interactions in the Caulobacter bacterium. First, we develop a simple stochastic differential equation model that describes the mechanics of ParA/ParB interactions, which we simulate numerically. Next, we develop a continuous model using partial differential equations to follow ParA/ParB interactions along with ParA monomer dynamics in the cytoplasm.

2. Model assumptions

In this section, we describe the assumptions for both models examined in this paper. The model components are shown in Fig. 1. ParA is assumed to assemble into a bundle of linear filaments, or polymers, based on observations from Ptacin et al. (2010). A ParA bundle of filaments is anchored and extends from the new pole ($x=L$) to the vicinity of the old pole ($x=0$), as
ParB proteins are envisioned to organize into a large rigid nucleoprotein complex, Fig. 1. We do not distinguish between individual ParBs in the complex, but instead assume that the complex is composed of a dense array of ParBs that are bound to parS DNA. Each ParB protein in this complex has affinity and can associate with ParA filament dimers. Since the structure of ParA filaments is not well understood we simplify the binding interactions by assuming that ParB binders have affinity for the ParA filament lattices, with no additional preference for specific ParA dimer binding sites. If indeed there are specific binding sites on each ParA dimer for ParB binders then we require that this binding is weak with low activation barriers for transition between consecutive sites. This requirement is imposed in order to allow for a bound ParB complex to easily readjust its position on ParA filament lattices. In previous work for eukaryotic kinetochores (Shtylla and Keener, 2011), we have shown that strong specific polymer binding greatly hinders the ability of binder arrays to adjust to dynamic filaments. In the case of ParB, since the complex itself causes ParA depolymerization, strong individual ParA dimer binding creates high probability for ParB complex detachment, which does not agree with the fast ParB translocation observed in experiments (Ptacin et al., 2010; Schofield et al., 2010; Shebelut et al., 2010). The energetics of the ParA/ParB binding interactions are described by an explicit free energy function, $V$, which we specify for each model below.

We envision that a ParB complex is arranged so that it can make contacts with multiple fibers of the ParA bundle. Chromosome movement is accomplished by lateral sliding of the complex on the bundle filaments. The structure of the ParB complex could be similar to the proposed ParR sites that encircle dynamic ParB filaments in Escherichia coli (Gerdes et al., 2010). Note, however, that here we do not explicitly model any details about the arrangement of the individual ParB complex binders on the ParA bundle. Since ParB has affinity for and stimulates ParA ATP-ase activity, we assume that ParB can bind and then depolymerize a ParA filament at rate $\beta$.

ParB cannot move freely in the cell because it is attached to a chromosome copy through the parS site, as depicted in Fig. 1. Since the size of the replicating chromosome is considerable, ParB movement is resisted by structures found in the cytoplasm (Cabeen and Jacobs-Wagner, 2010). Further, since replication is concurrent with segregation, the moving chromosome copy remains connected to replication forks that can apply resistive force against new-pole-directed translocation. Accordingly, the ParB complex is assumed to be attached to a constant load $F$, which opposes movement. We note here that this load representation is highly simplified; a more realistic ParB load equation will be feasible once more is known about replication fork dynamics and chromosome structure during Caulobacter division.

Finally, we envision that a ParB complex that is attached to a ParA bundle diffuses on the ParA filament lattices. Thermal effects must be taken into account here, due to the assumed weak ParB binding to specific ParA dimer binding sites.

The common parameter values for the models are as follows. The length of the typical Caulobacter cell, $L$ is fixed at 3 $\mu$m (Ptacin et al., 2010; Schofield et al., 2010; Shebelut et al., 2010). The effective drag coefficient for the ParB complex, $T$, is calculated based on the Stokes drag coefficient formula $T = \frac{6\pi \eta R_B}{\eta} = 0.06 \text{ pN s/\mu m}$, with cytoplasmic viscosity $\eta = 2 \text{ mPas}$ and $R_B = 1.5 \text{ \mu m}$, the radius of the sphere representing the ParB complex and the chromosome. The size of the ParB sphere is chosen in order to accommodate for ParB being connected to portions of the replicating chromosome copy, which in turn is partially tethered to unreplicated chromosome regions. The effective horizontal length of the ParB complex that is populated by binders, $L_B$ is estimated at 0.15 $\mu$m. The drag coefficient for ParA dimers and monomers is $T = \frac{6\pi \eta R_A}{\eta} = 2 \times 10^{-4} \text{ pN s/\mu m}$ with $R_A = 5 \text{ nm}$, the radius of the sphere representing the protein (Ringgaard et al., 2010). The diffusion coefficients for the ParB complex and ParA proteins are calculated from the Einstein relation, $D = k_B T / \eta$. A load force, $F = 1 \text{ pN}$ is included in both models, as detailed below. The rest of the parameters are specified when we describe each model.

3. Discrete model for ParB movement

In this section we develop a simple stochastic differential equation (SDE) model that addresses how a growing or retracting ParB bundle of filaments can move a ParB complex. In Fig. 2 we show a diagram of the discrete model setup. The assumptions made to construct this model are as follows. We represent a Caulobacter cell by a rectangular lattice with length $L$ and width proportional to the number of ParA filaments present in the cell. The ParB complex, represented by a rigid box of length $L_B$, is shown projected onto the ParB bundle in Fig. 2. The SDE model equations track the longitudinal or $x$-axis displacement of the ParB complex and ParA bundle tips.

In this model, ParB displacement on the $x$-axis is controlled by two forces: (1) a white noise forcing term due to thermal fluctuations of ParB, and (2) a deterministic force that arises due binding between ParB binders and ParA filaments. Binding between ParB and ParA is energetically favored. Thus, a decrease in the system free energy is achieved when more ParB binders make contact with ParA filaments. Since ParB is assumed to be densely packed with binders, we ignore the position of specific ParB binders relative to the ParA bundle. Instead, the binding force on the complex can be calculated if we know how much overlap there is between ParB and ParA filaments. For the overlap between ParB and ParA, we must know the configuration of the ParA bundle at any given time. Thus, for each ParA polymer $i$ in the bundle, we keep track of the position of the filament tip, $x_{\text{tip}}(i)$ and we also track the position of the ParB complex edge denoted by $x_c$, see Fig. 2. The total overlap between ParB and ParA

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Fig. 2. A diagram of the discrete Caulobacter model. The cell is envisioned as a rectangular lattice with length $L = 3 \mu$m. Multiple ParA filaments are aligned next to one another. The ParB complex (gray box) binds the ParA filaments but also removes ParA monomers from the filament tips.
filaments, \( A(x, x_{\text{tip}}(i)) \) is calculated as

\[
A(x, x_{\text{tip}}(i)) = \sum_{i}^{n} \max (x + L_b - x_{\text{tip}}(i), 0),
\]

where \( n \) is the total number of ParA polymers in the bundle. Note that by construction \( A \) has units of length.

From our binding assumptions we deduce that if \( A \) increases, then the ParB system free energy decreases, since more ParB binders can make contact with the filaments. However, the size of the ParB complex is necessarily finite, so the ParB binders will eventually be all occupied. Given the definition of the overlap variable \( A \) in Eq. (1), saturation of binders takes place when \( A = A^* = nL_b \). Consequently, if \( A > A^* \), the ParB system does not experience a decrease in free energy, i.e., there is no bias for ParB to increase the overlap with ParA. In accordance, the system free energy produces a bias for more overlap in this model provided \( 0 < A < nL_b \).

In view of our assumptions for binding, we construct a potential well function \( \Psi(A) \),

\[
\Psi(A) = \left\{ \begin{array}{ll}
-aA, & 0 \leq A < A^*, \\
-aA^*, & A \geq A^*.
\end{array} \right.
\]

The binding force \( \Psi'(A) \) reads

\[
\Psi'(A) = \left\{ \begin{array}{ll}
-\partial \sum_{i}^{N} x_{\text{tip}}(i), & 0 \leq A < A^*, \\
0, & \text{otherwise}.
\end{array} \right.
\]

The parameter \( a \) is measured in pN and it represents the ParB binding energy per unit length. The function \( z_{[x, x+L_b]} \) is an indicator function defined as

\[
z_{[x, x+L_b]} = \begin{cases} 
1, & x_{\text{tip}}(i) \in [x, x+L_b], \\
0, & \text{otherwise}.
\end{cases}
\]

Observe that the binding force term \( \Psi'(A) \) is dependent on the density of filament tips present in the overlap at a given time. Thus, if the total density of tips in the overlap increases, then the ParB complex will feel more force in response.

The total binding energy felt by a fully attached ParB complex in this model is given by \( anL_b \). The energetics of ParB/ParA binding interactions are currently not known, so we estimate the value of the parameter \( a \). Unless otherwise stated, for the discrete model simulations we use \( a = 1 \) pN. In Caulobacter cells, a ParB/ParS complex is estimated to have around 500 binders (Lim, 2011) which gives us 0.7 k_BT of binding energy per binder. Other choices for the value of the binding parameter \( a \) are discussed in Results.

An arrangement of tip positions for the discrete model is shown in Fig. 2. An important point to highlight here is that in this model the vertical position of a tip is not important. This is because when we calculate the ParB/ParA overlap, \( A \), we only keep track of the position of a tip along the \( x \)-axis and make no distinction in the overlap contribution of tips with respect to their position on the \( y \)-axis. Therefore permutations of the filament tip indices do not affect ParB binding forces. For illustration purposes, when we present results from the discrete model, we will typically follow two tips in the bundle: (a) a leading tip \( x_{\text{tip}}(i) = x_{\text{leading}}(i) = x_{\text{tip}}(0) \), which corresponds to \( x_{\text{tip}}(1) \) in Fig. 2, (b) a trailing tip \( x_{\text{tip}}(i) = x_{\text{trailing}}(i) = x_{\text{tip}}(0) \), which corresponds to \( x_{\text{tip}}(5) \) in Fig. 2. We can easily construct a ParA filament density function \( d(x) \) by counting the number of ParA filaments located at \( x \). The distance between the leading tip and the trailing tip gives us a measure of how fast \( d(x) \) varies in the horizontal direction. Small distances between leading and trailing tips cause the ParA density to have sharp edges, whereas large distances in extremal tip positions produce a more gradual transition in filament densities along the horizontal axis. Clearly, there are many combinations of filament lengths that can be used to initialize the problem. We choose “smooth” initial filament tip positions in order to match the initial conditions for the continuous model in the next section. Specifically, after rearranging in descending order, our initial configuration has \( x_{\text{tip}}(i) - x_{\text{tip}}(i+1) = 2L_b \), where \( \delta_m \) gives the length of the ParA dimer/monomer.

With the above assumptions in hand, we are now ready to write the model equations. The Langevin equations for the ParB and ParA tip locations read

\[
dx = \frac{1}{k_B} (\Psi'(A) - F) dt + \sqrt{2k_B} dW_t, 
\]

\[
dx_{\text{tip}}(i) = \delta_m n_{\text{par}}(i,t) + z_{[x_{\text{tip}}(i), x_{\text{tip}}(i)+L_b]} \delta_m n_{\text{par}}(i,t) - \delta_m n_{\text{par}}(i,t), 
\]

where \( n_{\text{par}}(i,t) \) and \( n_{\text{par}}(i,t) \) are independent homogeneous Poisson processes for each tip \( i \), with amplitudes \( \delta_m \) and ParA polymerization/depolymerization rates \( \alpha, \beta, \) and \( \beta_0 \). We assume, for the rest of this paper that ParA dimer addition/removal rates have constant values. The specific rate values used in our simulations were chosen such that the model ParB movement velocities agree with experimental data reported in Ptcin et al. (2010), Schofield et al. (2010), and Shebelut et al. (2010). Finally, \( W_t \) is standard white noise applied to the ParB complex.

The model consists of \( n + 1 \) equations in total, \( n \) equations for the positions of the \( n \) tips, and one equation for the position of the ParB complex. Unless otherwise stated, we consider dense ParA bundles with \( n = 10 \). At the boundaries \( x = 0 \) and \( x = L \), \( x \) is fixed to \( x = 0 \) and \( x = L \) to represent the capture of the ParB complex by proteins such as PopZ at the cell poles (Schofield et al., 2010). Further, we highlight that for this first model we have made some additional simplifying assumptions. Specifically, we have assumed that ParA monomers are abundant and well mixed in the cytoplasm so that the polymerization rate is not ParA monomer concentration-dependent. Further, the dimerization reaction of ParA in solution is assumed to be rapid compared to polymer growth, and we also ignore any TipN sequestration effects on ParA monomer concentration in the cytoplasm. The contributions from ParA monomer diffusion, ParA dimerization, and TipN sequestration are examined in the continuous model, which we discuss in the second part of this paper.

3.1. Discrete model results

The model equations given by Eqs. (5) and (6) are simulated numerically. In Fig. 3A we show two typical solution trajectories for \( x_c \) and \( x_{\text{tip}}(1) \), when ParA filaments are depolymerizing (\( x = 0 \)).

As can be seen from the plot in Fig. 3A, the solution trajectory for \( x_c \) experiences both white noise and a binding force which guides the complex toward the new pole at \( x = 3 \) \( \mu \)m. For the trajectories shown in Fig. 3, we observe that at least one ParA bundle filament \( x_{\text{tip}}(1) \) is located on average between \( x_c \) and \( x_c + L_b \), which indicates that all the ParB binding sites are not saturated and \( A < A^* \). Hence, the \( \Psi' \) forcing term biases the motion of \( x_c \) toward the new pole to increase the overlap. On the other hand, the tip position \( x_{\text{tip}}(1) \) experiences jumps in position due to the Poisson noise terms in the tip Langevin equations. Since the polymerization rate is set to zero for this simulation, only depolymerization jumps occur and the tip moves closer to the new pole as monomers are removed.

In Fig. 3B we show histograms for ParB positions, \( x_c \) with \( \beta_0 = 0.5 \text{ s}^{-1}, \beta = 0.05 \text{ s}^{-1}, \) and \( \alpha = 0 \text{ s}^{-1}. \) From the histograms in Fig. 3B, we observe that as time progresses the peaks of the ParB distributions shift toward the new pole, indicating that on average the binding drift term in Eq. (5) is pushing the complex to increase overlap with ParA filaments. On the other hand, we also notice a decrease in the peaks and an increase in the tails of
the distributions, which indicates that over time there is higher variability in ParB positions. We can get a better idea about the behavior of our model if we plot the average positions for both ParB and two representative ParA tips.

In Fig. 4, we show the average values for $x_A$ and ParA tips, $x_{tips}(10) = x_{tips}(5)$ and $x_{tips}(5)$ obtained from simulations with $\beta_0 = 0.5 \text{ s}^{-1}$, $\gamma = 0 \text{ s}^{-1}$ and varying $\beta$ values. The plot of position averages in Fig. 4A indicates that the ParA tips and the ParB complex closely track one another as they both approach the new pole, when $\beta \ll \beta_0$. Because ParB advances toward the new pole during these simulations, we deduce that the overlap remains, on average, under $A^*$ and the binding drift in the $x_c$ equation pushes firmly in the direction of the new pole. In Fig. 4B, where $\beta = \beta_0$, we see that the distance between the average positions for the leading tip, $x_{tips}(10)$ and trailing tip, $x_{tips}(5)$ quickly increases over time. A slight increase in the distance between the average leading and trailing tip positions also appears in Fig. 4A. This indicates that ParA density edges lose sharpness over time when interacting with ParB that operates with $\beta > 0$. This property can be understood if we refer back to our binding well function $\Psi$. As soon as ParB latches on to the ParA bundle, the binding energy well $\Psi$ pushes $x_c$ to maximize overlap to $A^*$, thus forcing the ParB complex to sit slightly ahead of the tips in order to fill as many binding sites as possible. This ParB position with respect to the ParA bundle unavoidably leaves some ParA tips out of the ParB overlap. The tip positions located the closest to ParB, such as $x_{tips}(10)$, consequently experience $\beta + \beta_0$ jump rates as opposed to $\beta_0$ for the tips that are left behind by ParB, such as $x_{tips}(5)$. The difference in depolymerization rates for the ParA tips creates a situation where there is a bias for tips moving away from one another over time (spreading out). This property is highlighted in panel B of Fig. 4, where there is a fast increase in the distance between the average $x_{tips}(5)$ and $x_{tips}(10)$ followed by large standard deviations for $x_{tips}(5)$ and $x_{tips}(10)$. On the other hand, as the ParA tips relocate, ParB distributions also quickly become more variable and experience erratic motion ahead of the trailing ParA filament tips, particularly when $\beta = \beta_0$, Fig. 4B.

The change in tip arrangement of the ParA bundle feeds back into the movement of ParB in two ways. First, as the tips spread out, the region for which $A < A^*$ on the ParA bundle edge increases, thus ParB can inch further forward toward the new pole. Second, the spreading of the tips on the ParA bundle lowers tip densities at a position $x$ on the bundle so $\Psi$ decreases. Therefore, diffusion slowly takes over other forces in Eq. (5), increasing the variance of ParB positions sitting on the ParA bundle, shown by the increasing ParB standard deviations in Fig. 4. Larger tails in the ParB distributions cause more variation in depolymerization rates on the ParA bundle filaments, which in turn results in increased variation in the positions of the ParA tips.
In conclusion, in this model, over time ParB binder and ParA tip distributions become less focused, and the standard deviations increase for both $x_c$ and $x_{tip}(t)$.

Super-resolution imaging in Ptacin et al. (2010) indicated that there are cells which can have ParA filaments trail ParB as it approaches the new pole. It is not clear what the density of the bundles is in these dividing cells. If the bundles are composed of multiple filaments ($n \geq 10$), then we predict that trailing ParA will be observed when ParB-mediated depolymerization is comparable to the natural ParA filament depolymerization rate, since for $\beta \approx \beta_0$, on average, $x_{tip} < x_c$. However, in these cases we also expect to see the ParB movement become more erratic as the new pole is approached due to the spreading of the ParA filament tips along the horizontal direction. Since movement reversals are not reported in Ptacin et al. (2010), we do not favor the case where $\beta \approx \beta_0$ as the primary mechanism for directed ParB motion. Instead, for these cases it is possible that some ParA filaments never make contact with ParB ($\beta_0 = 0$), while the rest of the bundle moves with $\beta_0 > \beta$.

Finally, we test the dependence of ParB positions on ParA bundle dynamics by simulating the model when the ParA bundle is in a polymerizing state with $x > \beta_0$. This state corresponds to a rescue event, where a new ParA dimer can be added to the tip and thus stop the further removal of hydrolyzed ParAs from the polymers. Note that there is an implicit assumption of ParA monomer and ATP abundance in the cytoplasm for rescue to occur. It is not clear what the growth and shortening dynamics of ParA bundles are during chromosome translocation. However, in Ringgaard et al. (2010) cells displayed catastrophe-like ParB-independent depolymerization of ParA, and in Ptacin et al. (2010) it was shown that ParA experienced ATP-dependent polymerization in vitro. These experiments raise the possibility that ParA filaments can experience both depolymerization and polymerization during division. Depolymerization and rescue events have been observed for ParM filaments in E. coli, where these filaments continuously transition between states of rapid growth and disassembly (Thanbichler and Shapiro, 2008; Garner et al., 2004).

A plot of the average positions for ParB and ParA tips when ParA is polymerizing is shown in Fig. 5.

As can be seen from the plot in Fig. 5A, when $x > \beta_0 > \beta$ the ParA filaments grow toward the old pole despite ParB binding and depolymerization. Since the polymerization rate is faster than depolymerization, ParB binders quickly become saturated ($A = A^*$), so the $x_c$ motion is solely controlled by the balance between $F$ and thermal motion, which quickly creates large tails in ParB distributions and ParA tip distributions. However, the load $F$ operates to oppose the motion of ParB toward the new pole, so as the ParA bundle grows, the ParB complex is eventually pushed to the ParA edge due to the load. As a result, when the ParA bundle is polymerizing the ParB complex will follow, thus further confirming that ParB movement is directly dependent upon ParA polymer dynamics.

In Fig. 5B we plot model solutions when $x - \beta_0 = \beta$. In this case the total depolymerization rate of ParA tips that overlap with ParB matches the ParA polymerization rate. For the ParA tips which are in contact with ParB there is no net movement due to polymerization/depolymerization because $x = \beta + \beta_0$. However, the ParB complex, driven by $\Psi(A)$, will try to relocate on the bundle in order to increase $A$. This ParB motion leaves some ParA tips behind, which start growing as soon as they lose overlap. In Fig. 5B we see that indeed the distance between $x_{tip}(5)$ and $x_{tip}(10)$ is much larger in Fig. 5B than in Fig. 5A. This large gap in tip positions is reflected in a quick spreading of ParB distributions over the ParA polymer lattices as the complex struggles to push toward the new pole by holding on to a few tips with large $A$. As a result of the erratic ParB motion, polymerization overrides depolymerization and slowly moves the tips and the ParB complex toward the old pole.

The ParB movement seen in this model when $x > \beta_0$ gives us an idea of what happens when ParA polymerization rates are not tightly controlled. ParB segregation to the new pole directly depends on how fast ParA filaments grow or shrink. More specifically, when ParA filaments manage to grow toward the old pole, ParB fails to segregate the chromosome to the new pole. Because the pool of monomers in this discrete model is assumed to be large enough to allow polymerization, with enough monomers, the ParA tips will grow pushing ParB to the old pole. Since the total pool of ParA monomers in bacterial cells is constant (Schofield et al., 2010), the growth of polymers shown in this discrete model is not physiologically relevant. However, the simulations with $x > 0$ allow us to correlate ParB movement with ParA filament dynamics. From the discrete model results we expect that the observed erratic motion with poleward trips of ParB when $x > \beta_0$ along with ParA filaments appearing behind ParB will be a prevailing feature of movement when ParA is allowed to freely polymerize. Indeed, in $\Delta tipN$ experiments ParA polymers grow behind ParB and ParB shows stalled and frequent backward movement (Ptacin et al., 2010; Schofield et al., 2010). Based on our model results, we suggest that in $\Delta tipN$ cells the observed ParB motion can be explained by allowing some ParA filaments in the bundles to be in polymerization mode due to the

Fig. 5. Average ParB and ParA tip positions versus time for $x = 1 \text{ s}^{-1}$. (A) $\beta = 0.05 = \beta_0 = 0.5 \text{ s}^{-1}$. ParA tips, $x_{tip}(5)$ and $x_{tip}(10)$ grow quickly toward $x = 0$ and overcome ParB-mediated depolymerization action. The ParB complex, $x_c$ follows the growing ParA edge back to $x = 0$ and segregation fails. (B) $\beta_0 = \beta = 0.5 \text{ s}^{-1}$. For larger $\beta$, some ParA tips are depolymerized by ParB. This creates larger deviations for $x_{tip}(5)$, $x_{tip}(10)$ and subsequently $x_c$. Both ParB and ParA tips move toward $x = 0$, on average. Each point in the plot is obtained by averaging 500 solution trajectories of Eqs. (5) and (6). The plot bars mark standard deviation.
high concentration of free ParA monomers in solution. High ParA monomer concentration in the cytoplasm can be justified by the removal of TipN-mediated accumulations of ParA monomers at the new pole (Ptacin et al., 2010; Schofield et al., 2010). This model prediction removes the need for the nucleation of ParA filaments from the old pole, as proposed by Schofield et al. (2010), in order to cause ParB movement reversals.

3.1.1. ParB binding strategies

The SDE model was primarily constructed in order to test and evaluate various ParB binding strategies with a bundle of dynamic ParA filaments. Unlike the PDE model we present next, the SDE model can be easily adapted to study segregation of ParB complexes engaging with ParA bundles of arbitrary thickness ($n \geq 1$). In this section, we discuss the effects of ParB binding strength on chromosome translocation patterns.

Two limiting cases for binding are presented in Fig. 6, where ParB interacts with a bundle that has made no prior contact with the complex (i.e., no natural depolymerization $\beta_0 = 0$). In Fig. 6A, we show the case when the energy for ParB association with ParA filaments is weak ($\alpha = 1 \text{ pN}, \text{as before}$). Since the complex is engaged with a thick bundle, the amount of free energy drop gained by engaging the ParB complex with an individual ParA filament is relatively small. As a consequence, the complex cannot remain committed to depolymerizing a single filament. Instead, thermal motion causes ParB to return to various ParA filament tips as it also struggles to depolymerize the bundle. In this case, the ParB complex remains committed to the bundle edge, which is desirable. However, increasing $\beta$ here does not cause the bundle edge shift to a new position, instead it only speeds up the spread of the tips. As noted in the previous section, the spreading effect of ParA tips is due to the bundle being composed of multiple filaments and ParB trying to maintain overlap by associating weakly with each ParA filament. The addition of ParA filament depolymerization with rate $\beta_0$ naturally counteracts tip spreading and focuses ParB movement so that there is directed translocation, on average, toward the new pole.

In Fig. 6B, we increased binding affinity to $20 \alpha$. As shown by the average position plots, stronger lattice binding for each ParA filament allows ParB to quickly and easily move while it depolymerizes the leading ParA filament. However, there is a drawback since in this case the majority of the filaments are left behind. Further, model simulations showed that as the binding affinity parameter is lowered from $20 \alpha$ to $\alpha$, the complex moves by depolymerizing different proportions of ParA filaments from the bundle, while also leaving a fraction of the bundle undisturbed. The features of ParB translocation displayed in Fig. 6A and B arise due to the dense nature of the ParA bundles. Indeed, if the bundle is composed of a single filament ($n = 1$), Fig. 6B (inset), model simulations show that strong ParB lattice binding is sufficient to allow ParB to move to the new pole. Clearly, for a single ParA filament natural depolymerization is not necessary to focus the ParB complex on the depolymerizing filament edge as shown by the overlapping average positions, $x_A$ and $x_{\text{tip}}(1)$ in Fig. 6B (inset).

However, based on the experimental evidence indicating that ParA organizes into clouds in these cells (Fogel and Waldor, 2006; Schofield et al., 2010; Shebelut et al., 2010), we expect that ParA filaments are organized into thick bundles made up of multiple filaments and chose a small in order to force ParB to remain committed to the ParA bundle edge. Yet, as we have seen from our results so far, in this case natural depolymerization ($\beta_0 > 0$) is necessary in order to prevent ParB from experiencing significant movement reversals.

3.1.2. Discrete model conclusions

In conclusion, we used our discrete model to test various scenarios for ParB/ParA interactions. We showed that if ParB clouds retract with ParB holding on at the edge, then ParB binding must be adapted to allow for multiple filaments to depolymerize at the same time. We saw that one way to commit ParB to the edge was through weak binding of the complex to each ParA filament. However, in the dense bundle case where ParA does not experience some ParB-independent depolymerization, the weak association of ParB with each individual filament counteracts fast ParB translocation. We showed that for various parameter combinations this model can reproduce some key characteristics of ParB/ParA interactions that are seen in Caulobacter cells. From this first simple model we predict that fast ParB-mediated depolymerization of ParA filament bundles is not necessary to drive chromosome motility in Caulobacter. Instead, independent ParA polymerization/depolymerization coupled with biased diffusion of ParB on the ParA lattice are sufficient to produce directed chromosome movement.

A drawback of the discrete model is that it becomes computationally expensive to follow the dynamics of each ParA monomer in the cytoplasm. The cytoplasmic ParA monomer concentrations affect the ability of ParA filaments to polymerize, and the discrete model results indicated that chromosome movement direction is sensitive to...
to the ParA bundle polymerization/depolymerization rate balance. Thus, we expect a connection between ParB motility and ParA monomer concentrations, and thereby it is important to directly track ParA monomer dynamics in the cytoplasm and their effects on ParA bundle growth. Proteins which interact with ParA monomers in the cell, such as TipN, would be a natural candidate for the control of ParB movement. We show in the next section that a continuous model can be built to address chromosome movement control by TipN.

4. Continuous model for chromosome segregation

In this section, we use some of the results from the discrete model for ParB/ParA interactions in order to develop a continuous model which tracks the complete segregation apparatus of Caulobacter. We start by listing assumptions that are specific to the continuous model.

A Caulobacter cell is assumed to be a cylinder of length \( L \), Fig. 7A. Since ParB experiences little motion along the width of the cylinder and the ParA bundles also localize across the length of the cell, we ignore any ParB movement or ParA dynamics along the width of the cell. Thus, for this model we keep track of ParB location and ParA concentrations along the \( x \)-axis which starts at the old pole (\( x=0 \)) and ends at the new pole (\( x=L \)), as depicted in Fig. 7A.

The ParA bundle has varying filament densities along the cell, which must be properly projected on the \( x \)-axis. In this model we track the ParA bundle filament cross sectional density denoted by \( A(x) \). So, the function \( A(x) \) gives the total number of ParA filaments per cell cross sectional area at position \( x \) (i.e., it has units \( \mu \text{m}^{-2} \)). The main point is that \( A(x) \) is assumed to be a continuous function of \( x \), so the discrete nature of tip ends is smoothed out, such an assumption is not appropriate unless the total number of ParA filaments in the bundle is relatively large. For the rest of this paper, we set \( A(x) = \bar{A}(x)/\bar{A}_0 \), where \( \bar{A}_0 \) is the maximal number of filaments in a cross-section in the cell at \( t=0 \). We initialize the ParA bundle density with a smoothed step-function, as depicted in Fig. 7B.

We now define the binding energy well function, \( \Psi \). As before, we assume that each ParB binder element has affinity for a ParA bundle filament. Since the complex is densely populated by binders, we do not consider each ParB binder attachment event individually, but instead keep track of the binding interactions occurring per unit length of ParB. Thus, for a ParB complex engaged with all the filaments in the cross-section (\( A=1 \ \mu \text{m}^{-2} \)), we assume there is a free energy drop \( -a = -\bar{a} \gamma \), where \( a \) is the energy per unit length of ParB and \( \gamma \) is the cell cross-sectional area. However, at a position \( x \), ParB encounters and interacts with various filament density proportions, so that the total energy per unit length of ParB is given by \( \psi(x) = -a A(x) \). Then total binding energy of the ParB complex interacting with a ParA bundle is calculated as the sum of binding energy over the length \( L_B \) of the ParB complex. So we write

\[ \Psi = \int_{x_{-L}}^{x} \psi(x) \, dx, \]

\[ \Psi = -a \int_{x_{-L}}^{x} A(x) \, dx. \]

Note that in the above expression there is an inherent assumption that ParB binders are sufficiently dense on the complex to warrant integration. Also, \( x \) here marks the position of the front edge of the ParB complex (equivalent to \( x_B + L_B \) in the discrete model). Since the length of the ParB complex is small compared to the length of the cell (\( L_B \ll L \)) we can further simplify Eq. (8) to obtain

\[ \Psi(A) = -a L_B A(x). \]

Observe that the expression in Eq. (9) gives us a direct relationship between the cross sectional density of ParB bundle filaments and the energy of binding. Indeed, if the density of filaments in the bundle is increased, then the ParB complex will reach more filaments and the free energy is lowered accordingly. However, the total amount of energy arising from the binding interaction must be capped off since the binders on ParB will eventually be all occupied. In order to achieve this we construct a saturating function:

\[ K(A) = \begin{cases} A, & 0 \leq A < A^*, \\ A^*, & A \geq A^*. \end{cases} \]

and the binding energy now reads

\[ \Psi(A) = -a L_B K(A). \]

We choose \( A^* = 1 \ \mu \text{m}^{-2} \). Note that the saturation of the energy will become relevant when we consider filament nucleation at the new pole, which can cause \( A(x) > 1 \ \mu \text{m}^{-2} \).

Fig. 7. Continuous model components. (A) A diagram of the filaments and ParB complex for the continuous model. (B) The function \( A(x) \) is a smoothed step function and it represents the cross-sectional density of the ParA bundle along the length of the cell. The density transition point is at \( x_A = 0.5 \mu \text{m} \). The total amount of binding energy, \( \Psi \) is dependent upon the total number of filaments that a ParB complex can bind.
A note is in order about the binding force term $\Psi'(A)$ (‘ indicates derivative with respect to $x$). The binding force in this model is directly proportional to $A$. The term $A$ is a measure of the density of exposed ParA filament tips along the $x$–axis. Thus, binding forces on the complex increase if there are more exposed filament tips. Finally, the specific value of the energy parameter ‘$a$’ for this model, is chosen such that the binding force term, $\Psi'$ in the $p_B$ equation agrees with the corresponding term in the discrete model (note that this value depends on the functional form of $A(x)$, for the simulations $a = 20 \text{ pN} \cdot \mu \text{m}^2$).

ParA monomers are assumed to undergo an ATP-dependent dimerization reaction in solution. We track the concentration of monomers with $A_M(x)$ and the concentration of dimers with $AD(x)$. When there is no monomer sequestration by other proteins, we enforce conservation of total volume using

$$\text{total bundle volume} + \text{total monomer/dimer volume} = \text{constant},$$

$$\int \left( \frac{A_M(x) + A_M(x) + 2AD(x)}{dx} = AM_f L, \right)$$

where $AM_f$ is the total concentration of monomers in the cell.

Finally, TipN has been proposed to be an essential component needed to maintain ParB directionality during cell division (Schofield et al., 2010). TipN affects ParA dynamics in the cell by accumulating ParA monomers at the new pole. It is not clear how TipN interacts with the ParA proteins so we explore two modes of TipN activity: (1) sequestration and (2) nucleation. For the first case, TipN is assumed to bind and thus remove ParA monomers from the cytoplasm so that they cannot return to the ParA bundle (Schofield et al., 2010). A slightly different scenario is proposed in Ptacin et al. (2010), where it is suggested that TipN proteins facilitate ParA filament nucleation at the new pole. The molecular details of TipN-mediated ParA nucleation are not known, so we retain a simplified approach for the nucleation model. Therefore, we implement the second scenario by considering the model with the additional assumption that all TipN-sequestered monomers are added back to the ParA bundle. Specifically, a monomer is assumed to be added to the bundle by TipN at some position $x$ and then the monomer serves as a new filament tip, which in addition to growing or shortening with the same rates as the rest of the bundle filament tips ($\beta_{tip}(x)$), it can also grow when more ParA monomers are added by TipN action. A TipN-nucleated polymer may also undergo depolymerization if in contact with ParB, like the rest of the ParA bundle.

Based on the above assumptions we write the following system of equations for interactions between ParA and ParB in a dividing Caulobacter cell:

$$\frac{\partial A}{\partial t} = 2x|A_x|AD - \beta_0|A_x| + \kappa_n k_{tip}(x)AM - \frac{\beta_l|A_x|}{K_a + |A_x|} p_B(x) dx,$$

$$\frac{\partial p_B}{\partial t} = -\frac{1}{\nu_B x} ((-\Psi'(A) - F)p_B) + D_A \frac{\partial^2 p_B}{\partial x^2},$$

$$\frac{\partial AM}{\partial t} = -2k_N AM^2 + \beta_0|A_x| - k_{tip}(x)AM + \frac{\beta_l|A_x|}{K_a + |A_x|} p_B + D_A \frac{\partial^2 AM}{\partial x^2},$$

$$\frac{\partial AD}{\partial t} = k_N AM^2 - \beta_0|A_x| AD + D_A \frac{\partial^2 AD}{\partial x^2}.$$
the model equations are

\[
\frac{1}{\alpha_m} \frac{\partial \rho}{\partial t} = \frac{\partial}{\partial x} \left[ \rho \left( A + \beta_0 \rho \right) - \frac{\beta L_0}{K_0 + \rho} \right],
\]

\[\frac{\partial p_B}{\partial t} = \frac{1}{v_B} \left( (-\Psi^\prime (A) - F) p_B + D_{p_B} \frac{\partial^2 p_B}{\partial x^2} \right),\]

\[\frac{\partial AM}{\partial t} = -2 \frac{\partial}{\partial x} \left[ \rho \left( A + \beta_0 \rho \right) + \frac{\beta L_0}{K_0 + \rho} \right] - D_A \frac{\partial^2 AM}{\partial x^2}.\]

We note that in Eqs. (22) and (24) the polymerization rate is modified to reflect that ParA dimerization is not included in the polymerization term.

In Fig. 9A–C we show model solutions for a depolymerizing ParA bundle \((\beta_0 = 0.5 \text{ s}^{-1}, x = 0)\) and varying depolymerization rates, \(\beta\) for the ParB complex.

As can be seen from the plots for \(p_B(x)\) and \(A(x)\) in Fig. 9A, when \(\beta < \beta_0\), the ParB distributions closely follow the ParA bundle edge as it depolymerizes toward the new pole. The ParA bundle density, \(A(x)\) shows almost wave-front movement with edges that slightly lose steepness over time. The reason for this smoothing is the same as in the discrete model. The ParB complex sits at the bottom of the \(\Psi\) well, which localizes close to \(A(x) = 1\). The positioning of the ParB complex ahead of the ParA tips causes faster depolymerization close to \(A = 1\), as compared to \(A = 0\). This difference in the depolymerization rates of the ParA bundle

![Fig. 9](image-url). \(p_B(x)\) (red) and \(A(x)\) (blue) for depolymerizing (A–C) and polymerizing (D–F) ParA bundles. (A) \(\alpha = 0\) and \(\beta = 0.05 \text{ s}^{-1}\). The ParB distributions follow the ParA bundle closely since \(\beta < \beta_0\). (B) \(\alpha = 0\) and \(\beta = 0.1 \text{ s}^{-1}\). ParB distributions move slightly ahead of the ParA bundle edge. (C) \(\alpha = 0\) and \(\beta = 0.5 \text{ s}^{-1}\). ParB distributions move significantly ahead of the ParA bundle edge since the complex removes monomers with \(\beta > \beta_0\). (D) \(\alpha = 1 \text{ s}^{-1} \mu m^2\) and \(\beta = 0.05 \text{ s}^{-1}\). ParB distributions initially follow the ParA bundle closely and then slow down. (E) \(\alpha = 1 \text{ s}^{-1} \mu m^2\) and \(\beta = 0.1 \text{ s}^{-1}\). ParB distributions fail to segregate despite higher \(\beta\). (F) \(\alpha = 1 \text{ s}^{-1} \mu m^2\) and \(\beta = 0.5 \text{ s}^{-1}\). ParB distributions localize ahead of the ParA bundle edge, but also fail to segregate. Each solution is shown every \(t=1.2\) min for a total of 10 min of segregation time. For all panels \(\beta_0 = 0.5 \text{ s}^{-1}\). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
moves some ParA filament tips farther ahead of the bundle edge. The ParB complex distributions also experience stronger diffusion effects as $A(x)$ retracts, because as time progresses, $A_{n}$ decreases causing a decline in the magnitude of $\Psi$. This is shown by the smaller peaks and lower distribution tails of $p_{b}$ as it approaches the new pole in Fig. 9A. Note that due to the decline in $p_{b}$ peaks as ParB approaches the new pole, high loads $F$ eventually cause the ParB complex to detach. We thus expect that for a depolymerizing ParA filament bundle interacting with a ParB complex, the likelihood of ParB detaching under load increases as the ParB bundle gets shorter. This prediction is in agreement with the experimental observations and assumptions of Ringgaard et al. (2010) in *E. coli* plasmids. In *Caulobacter*, the increased detachment probability due to low ParB binding site densities at the new pole is remedied by PopZ, which has been shown to anchor ParB at the new pole to prevent movement reversals (Schofield et al., 2010).

In panels B and C of Fig. 9, we show results of our simulations with the same $\beta_{0}$, but with higher ParB-mediated depolymerization rates, $\beta$. These plots indicate that an increase in $\beta$ causes the ParB complex to depolymerize the front edge of $A(x)$ faster, thus creating a faster overall decay in $A_{n}$ over time. In response, the ParB complexes experience stronger diffusive effects with the $p_{b}$ distributions having lower peaks as compared to the distributions shown in panel A. The gentle linear indentations on the ParA bundle for panels B and C in Fig. 9, are due to large differences in $\beta_{0}$ and $\beta + \beta_{0}$. When there is faster monomer removal by ParB, more tips are exposed ahead of the ParA bundle edge to which ParB can bind and depolymerize. As a consequence, $p_{b}$ spreads at the front of the ParA bundle to bind the tips that have been quickly shortened. Due to the small constant $A_{n}$, ParB binds these sites with equal probability so the $p_{b}$ distributions attain an almost box-like shape. This feature is particularly prominent in panel C where the $p_{b}$ peaks spread over 0.5 $\mu$m ahead of the ParA edge. The shape of the ParB distributions for $\beta = \beta_{0}$ indicates that ParB complexes have equal probabilities of moving closer to the new pole or away from the new pole, so that ParB complexes experience erratic motion as they segregate.

From the plots in Figs. 9A and 8C we see that an increase in ParB rates works against the ParB complex. By quickly decaying the front edges of $A(x)$, ParB complexes can quickly advance toward the new pole without fully degrading the bundle, causing $p_{b}$ to spread its peaks and diffuse out ahead of the ParA bundle edge. We therefore conclude from these simulations that a ParB complex composed of multiple binders can hold on and move with a depolymerizing ParA bundle edge, provided that the ParB-mediated depolymerization rates are small compared to $\beta_{0}$. In light of the experimental data which show ParB closely trailing a depolymerizing ParA bundle edge with no movement reversals, we favor the hypothesis that ParB slowly depolymerizes the ParA filaments and waits for independent ParA depolymerization to move both the complex and the ParA bundle front. In this scenario ParB has a passive depolymerization role. The results obtained thus far are in complete agreement with our discrete model results.

Next, we performed simulations in the case when the ParA filaments are allowed to polymerize with $\tau > \beta_{0}$, i.e., when the ParA bundle is in polymerization mode.

Simulation results for polymerizing ParA bundles are shown in Fig. 9D–F. As can be seen from Fig. 9D, when $\beta < \beta_{0}$, the ParA bundle initially moves closer to the new pole until the monomer pool is large enough to allow for polymerization of the ParA bundle filaments. Then, the ParA bundle experiences growth toward the old pole with tips close to $A = 0$ growing the fastest due to no overlap with ParB. This ParA growth causes a fast decay in $A_{n}$ and a subsequent spreading of $p_{b}$ distributions due to diffusion. The end result is that ParB complexes with slow ParB-mediated depolymerization rates first move toward the new pole, then experience a significant slow down (almost stalling) followed by higher probabilities for toward and away movement from the new pole. In this case, it is clear that the natural depolymerization rate $\beta_{0}$ cannot rescue ParB from slowly depleting the concentration for ParA filament tips that it can bind to. The ParB complex thus fails to segregate. Given enough time, the ParA edge will decay and significantly decrease $A_{n}$, so the ParB complexes detach and move close to the old pole under the load $F$. Next, from the simulation results in Fig. 9E and F, we conclude that ParB fails to segregate if ParA monomers are quickly returned to the bundle independent on how fast ParB depolymerizes. The main difference with higher $\beta$ is that the $p_{b}$ distributions spread out farther and faster in the cell indicating that the ParB complexes experience highly erratic motion closer to the new pole. It is important to highlight that in all simulation results presented in Fig. 9D–F, ParA polymers always appear between the old pole and the ParB complex. These results are in agreement with experimental observations, where when ParA monomers are abundant in the cytoplasm (due to TipN deletion), ParB complexes experience frequent reversals in direction while ParA filaments appear in between ParB and the old pole (Ptacin et al., 2010; Schofield et al., 2010).

### 4.2. Chromosome segregation with TipN and ParA dimerization

So far by solving Eqs. (22)–(24), we have not taken into account that the ParA monomers, released due to depolymerization, are required to dimerize before returning to the ParA bundle. Recent experimental evidence seems to indicate that ParA monomers may undergo conformational changes before dimerizing in the cytoplasm (Vecchiarelli et al., 2010). This data suggests that there is a delay in the polymerization of ParA filaments once monomers are in solution. In this section, we introduce such a dimerization delay by simulating the full model, Eqs. (18)–(21), using a nonzero dimerization reaction rate, $k_{d} < \tau$. In Fig. 10, we show model solutions when dimerization is allowed and there is no ParA monomer interaction with polar proteins ($k_{\text{TipN}} = 0$).
From the $A(x)$ and $p_B(x)$ solutions in Fig. 10 we see that when dimerization is added, the ParB complex initially experiences slow motion toward the new pole. The ParB distributions in this case move closer to the new pole and retain their peaks better than in Fig. 9E, where no dimerization delay was present. However, even in this case when the dimer concentrations are sufficiently high, $A(x)$ grows toward the old pole. As a consequence, the ParB complex fails to segregate the chromosome in the same time frame as when ParA bundles are allowed to freely depolymerize. Thus, unless the dimerization delay is long enough to allow for full chromosome segregation (which from experiments seems not to be the case Vecchiarelli et al., 2010), the monomers in solution need to be somehow removed or recycled in locations that do not prevent ParB from moving with a depolymerizing ParA bundle. This monomer sequestration action is precisely what TipN is hypothesized to do.

To understand the role of TipN, we examine the case where TipN is assumed to sequester ParA monomers from the cytoplasm. To this end, we introduce TipN in the model Eqs. (18)–(21)

\[
A(x, t) = \frac{k_{\text{tip}}}{C_0} \frac{1}{A(x, t)^2} \\
p_B(x, t) = k_{\text{tip}} \frac{A(x, t)}{C_0} \\
\]

where $k_{\text{tip}}$ is the rate function $ctip(x)$ with $k_{\text{tip}} \neq 0$.

In Fig. 11A, we show model results of the TipN sequestration scenario. In this case we set $k_5 = 0$ in Eq. (18) to enforce that the monomers are not returned to the ParA bundle after being sequestered. As can be seen from the $p_B(x)$ and $A(x)$ solutions in Fig. 11A, the delay from ParA dimerization coupled with fast TipN monomer sequestration fully restore segregation of the Caulobacter chromosome in the cell. Thus, in this model TipN is essential for segregation.

Next, we examine the case where TipN not only sequesters monomers but also assembles them onto the existing ParA bundle so that dimers can be recruited and new ParA filaments are formed. We simulate our model Eqs. (18)–(21) with $k_5 = 1$ and the results are plotted in Fig. 11B.

The plots in Fig. 11B show that the introduction of the nucleation action of TipN creates new ParA bundle filaments close to the new pole, as marked by the increase of $A(x)$. TipN-generated filament tips also shorten/grow following the rates $\beta$, $\beta_0$, $x$ AM like the rest of the bundle. The ParB complex segregates the chromosome due to the delay in dimerization coupled with the monomer recruitment from TipN. We note that the main difference between the nucleation and the sequestration model forTipN operation has to do with the shape of the ParB probability density solutions, $p_B$. In the case when TipN only sequesters monomers, the shape of $p_B$ is identical to the distributions of depolymerizing ParA filaments in the previous section. Accordingly, the distributions diffuse out as the ParA tip density is decreased. In the case of nucleation, the tip density is kept high close to the new pole due to the addition of TipN nucleated polymers. Thereby, TipN nucleation keeps the $p_B$ distributions sharply focused on the ParA edge even when close to the new pole ($A_p$ is large close to the new pole as TipN nucleates). Thus our model predicts that the probability of late filament detachment is lowered when TipN nucleates ParA filaments at the new pole, providing a more robust segregation mechanism. Both TipN models restore segregation, with accumulation of either monomers or generation of polymers at the new pole. In both cases our model indicates that there is a large concentration of ParA protein at the new pole when TipN is working. These results are in agreement with experiments where the addition of TipN to ΔtipN cells restored segregation and accumulations of ParA proteins were observed at the new pole (Schofield et al., 2010). In the nucleation scenario, ParB segregation directionality is reinforced by the higher gradients of ParA polymer close to the new pole. We conclude by noting that our continuous model shows that both modes of TipN can work to restore segregation, which is to be expected since in both cases there is a common underlying mechanism of redirecting ParA monomer from the cytoplasm to the new pole.

5. Discussion

In this paper we have presented two models that explore mechanisms for the segregation of the chromosome copies of Caulobacter bacterium. In the first model, we put forward a simple mechanism for how a ParB complex can hold on to and move with
a polymerizing/depolymerizing ParA bundle. From our model we deduced that if ParB is allowed to have multiple binding interactions with the ParA bundle which depolymerizes with a natural depolymerization rate, movement of the ParB complex toward the new pole can be sustained. We showed that to reproduce experimental observations showing fast directed ParB motion while it is engaged with ParA, then ParB has to depolymerize ParA with rates that are much lower than the natural depolymerization rate of the ParA bundle filaments. Thus, we predict that the ParB complex plays a passive role in ParA bundle shortening. On the other hand, the velocity of ParB is directly dependent upon the ParA growth/shortening rates. We also showed that if polymerization is allowed in this simple model, segregation fails and the complex has an increased probability of experiencing significant backward motion toward the old pole.

In the second part of the paper, we presented a generalized continuous model which built on the discrete model results in order to capture ParB/ParA interactions and also followed the biochemical reactions that ParA monomers and dimers undergo once released in the cytoplasm. From this continuous model, we obtained similar results to the discrete model, where biased diffusion coupled with a retracting polymer bundle generated proper segregation of the ParB complex. Using the continuous model we can study the effects of dimerization delay on the system as well as the effects of TipN proteins acting at the new pole end. Our model supports a scenario where TipN action on ParA monomers controls ParA dynamics and thus consequently the polymerization rate of the ParA bundle. Since ParB movement is sensitive to ParA growth/shortening rates, this TipN action is sufficient to control the direction of ParB movement. These results are in agreement with experimental observations in Ptacin et al. (2010) and Schofield et al. (2010). We examined two modes of TipN operation in the cell and showed that both mechanisms could work to properly segregate ParB in a dividing cell. In the case when TipN could nucleate new ParA filaments into the bundles, we observed that ParB segregation was more robust due to higher ParA filament gradients close to the new pole.

An important aspect of the models proposed here is that binding interactions between ParB and the ParA bundle are quantified by the overlap between the complex and the filaments. This treatment reduces the dimensionality of the problem since we do not need to track the location and binding status of each individual ParB protein. Thereby, predictions can be made by following only a few variables.

Our mathematical models were constructed in order to examine the hypothesis that nonspecific ParB engagement to the ParA bundle is necessary to allow for ParB to move with dynamic cytoskeletal filaments. We found that this mechanism worked well in the case when ParB is engaged with thick dynamic ParA bundles, and in these cases ParB kept up with the edge of a retracting ParA cloud in agreement with experimental data (Fogel and Waldor, 2006; Schofield et al., 2010; Shebelut et al., 2010). In Ptacin et al. (2010), it was observed that in some cells ParA filaments stayed behind the translocating ParB complex, in contradiction with the ParA cloud dynamics observed by other groups, where ParA was reported to only localize between ParB and the new pole. We can explain this as due to ParB not encountering some ParA filaments at all during division, which can be caused by various geometrical constraints in these cells. Alternatively, we also found with the discrete model that when ParB is strongly associated with the lattice of a single ParA filament, then a significant portion of bundle ParA filaments will be left behind even if ParB has access to them, simply because the energy of ParA/ParB association is enough to allow ParB to move while depolymerizing only a small fraction of the bundle. Experiments that measure the binding energetics between ParB and ParA will allow us to distinguish between these two possible explanations for the appearance of trailing ParA filaments.

It is important to discuss the biological significance of the depolymerization rates for ParA bundles. We have found that ParB quickly translocates to the new pole by holding onto the edge of a degrading bundle provided that $\beta_0 \geq \beta \approx 0$; i.e., ParB moves by “passively” riding ParA depolymerization. However, one wonders what passive movement really means for ParB, in light of its stimulating action on the ATPase activity of ParA. We suggest that ParB-mediated depolymerization is a two step process composed of a hydrolysis step and a subsequent depolymerization step, so the values of $\beta$, $\beta_0$ have implications for both processes. Based on the observations of Ringgaard et al. (2010), we interpret $\beta_0$ as the depolymerization rate of ParA filaments that have experienced hydrolysis stimulation by ParB before the ParB commitment stage (for $t < 0$). On the other hand, $\beta$ measures a direct increment in hydrolysis speed and subsequent amplification of depolymerization rate upon additional contact of ParA with ParB. Thus, in the passive scenario ($\beta_0 \geq \beta$), ParB does not have a significant hydrolysis/depolymerization amplification effect, and movement is completely driven by depolymerization at rate $\beta_0$. On the other hand, when $\beta_0 = 0$, $\beta > 0$, ParB must both hydrolyze and depolymerize all filaments, while also maintaining overlap with a thick bundle. The dual task to remain bound to a thick bundle, while also mediating ParA hydrolysis with the slow rate $\beta$ causes ParB to lose directed motion. When $\beta_0 = \beta > 0$, ParB enhances initial hydrolysis and consequently amplifies some ParA tip depolymerization rates to $\beta_0 + \beta$, however, this creates difficulties with thick bundles when the amplification is significant, as described in the results. A key idea here is that for this depolymerization-driven movement to work, ParA dimer removal cannot be faster than the rate of ParB translocation between consecutive binding sites on the filaments. This can be achieved either by having very fast hydrolysis of ParA but a decoupled depolymerization event that happens with a slower rate, or having slow hydrolysis accompanied by immediate ParA depolymerization. Similar observations were made by Banigan et al. (2011), in which various hydrolysis and depolymerization rate ratios were tried in their simulations.

An important issue to consider is the rigidity of the ParB complex. Here, we opted to view the parS-associated ParB proteins as a rigid cluster of binding sites. Because of this rigidity, binding saturation for the complex can only be achieved if the ParB complex slides laterally on the bundle. Clearly, this sliding mediated by weak ParA filament binding was the precursor to frequent movement reversals seen for ParA bundles where $\beta_0 = \beta$. Based on the discrete model simulation results presented here, we predict that some of these reversal tendencies will be removed if the ParB complex is allowed to become more flexible, such that it can maintain overlap in the face of depolymerization. However, if the complex is too flexible, excessive stretching dilutes the number of binders that can be associated with a certain filament leading to slow translocation followed by complete detachment of the complex. Many of these issues were observed in Banigan et al. (2011), where ParB was composed of flexible polymers. We believe that the directed motion reported for bundles of various thickness in their study resulted from this assumed flexibility of the ParB complex.

It must be highlighted here that the discrete model and the corresponding continuous model agree because the bundle filament density for the discrete model is large. Therefore the results presented for both models are consequences of the assumption that ParA bundles are composed of multiple filaments and that filament densities change smoothly with respect to position. The nature of ParA bundles is not known, so a variety of scenarios are possible. We discussed how, in the $n=1$ case, our binding
hypothesis worked well independent of the relationship between $\beta_0$ and $\beta$. However, for thin bundles, discrete effects are significant and the continuous model does not apply.

Finally, an important assumption for our models is that ParA proteins are arranged into bundles of continuous filaments during division. However, it is not clear from the current experimental data whether ParA clouds seen in dividing cells indeed form such continuous polymeric arrays. In fact, it is possible that the ParA cloud is formed by a network of short dynamic ParA filaments. A key result from our model is that ParB movement is very sensitive to ParA filament dynamics. Therefore, if ParA does not form continuous filaments, we expect ParA movement dynamics to be altered due to the variations in ParA configuration. Furthermore, we have thus far assumed that a ParA complex has made initial contact with all ParA filaments so that depolymerization with rate $\beta_0$ is taking place in all filaments. The sequence of steps during the initial ParB/ParA interactions is not clear, thus scenarios where not all ParA filaments are activated into a catastrophe-like depolymerization state cannot be excluded. We hope to address these issues related to ParA bundle configuration and ParA depolymerization and their effects on ParA movement in future work.

An interesting aspect of DNA segregation in Caulobacter is the striking similarity between the ParAB segregation machinery and the mitotic spindle in higher eukaryotes (Bloom and Joglekar, 2008). We have previously shown that biased diffusion coupled to dynamic microtubule tips can produce movement of eukaryotic kinetochores (Shyially and Keener, 2011, 2010). Similarly, in the models of chromosome segregation proposed in this paper, we see that weak binding in conjunction with dynamic polymers and diffusion can produce directed movement of chromosome copies in bacterial cells. Furthermore, our model shows that by carefully regulating ParA filament dynamics with the help of polar proteins such as TipN, bacterial cells can control the direction of chromosome movement. A similar control theme appears in higher eukaryotes, where the modulation of microtubule dynamics at kinetochores can lead to a variety of chromosome movement patterns. Further, for Caulobacter, we have presented a study which takes into account multiple dynamic cytoskeletal filaments interacting with the chromosome, and for kinetochores detailed theoretical models have only been explored when one microtubule is associated with kinetochore structures. Kinetochores, however, are composed of a large number of binding complexes that engage with several dynamic microtubules at the same time (k-fiber bundles) (Cheeseman and Desai, 2008; Santaguida and Musacchio, 2009). The model we have proposed here may provide a platform for studying how binding interactions with multiple dynamic filaments can affect kinetochore movement.

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References


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