Self-organised segregation of bacterial chromosomal origins

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Abstract

In spite of much effort, many aspects of chromosome organisation and segregation in bacteria remain unclear. Even for *Escherichia coli*, the most widely studied bacterial model organism, we still do not know many of the underlying mechanisms. Like many other bacterial, the chromosomal origin of replication in *E. coli* is dynamically positioning throughout the cell cycle. Initially maintained at mid-cell, where replication occurs, *ori* are subsequently partitioned to opposite quarter positions. The condensin Structural Maintenance of Chromosomes (SMC), which is required for correct chromosome compaction and organisation, has been implicated in this behaviour but the mode of action is unknown. Here, we build on a recent self-organising model for the positioning of MukBEF, the *E. coli* SMC, to propose an explanation for the positioning and partitioning of origins. We find that specific loading of MukBEF onto the chromosome within the origin region, results in a non-trivial feedback between the self-organising MukBEF gradient and the origins, leading to accurate positioning and partitioning as an emergent property. We compare the model to quantitative experimental data of origin dynamics and their colocalisation with MukBEF clusters and find excellent agreement. Overall, the model suggests that MukBEF and origins act together as a self-organising system for chromosome segregation and introduces protein self-organisation as an important consideration for future studies of chromosome dynamics.

Introduction

The faithful and timely segregation of genetic material is essential for all cellular life. In eukaryotes the responsibility for chromosome segregation lies with a well-understood macromolecular machine, the mitotic spindle. In contrast, the mechanisms underlying bacterial chromosome segregation are much less understood but are just as critical for cellular proliferation. Nevertheless, a lot has been learned in recent years (see [1] for a review). In particular, the starting point for (bidirectional) chromosomal replication, the origin, has been found to have a crucial role in chromosome organisation and segregation. Not only is it duplicated and segregated first but its position defines other macrodomains [2].

The positions of genomic loci, including the origin, change as replication and segregation progress. Initiated at the chromosomal origin, replication proceeds bi-directionally down each arm of the chromosome. In slowly to moderately growing (1-2 hr generation time) *Escherichia coli* cells, the home positon of the origin, *oriC* (henceforth and in the

model, *ori*), in new-born cells is at mid-cell [3–5]. After 10-15 min of cohesion [6–8], duplicated origins separate and migrate, initially very rapidly, to opposite quarter positions, which become the new home positions for the remainder of the cell cycle [9,10]. Other genomic loci migrate sequentially to the replisome 'factory' at mid-cell [11,12] and subsequently segregate with similar dynamics [10]. However, in spite of extensive research, the forces underlying this behaviour are unknown.

Many bacterial species carry ParABS (Par) systems on their chromosomes [13]. First discovered in low-copy number plasmids, where they are required for correct partitioning, they also play a role in chromosome segregation. However, they are typically dispensable during normal growth [14–17] (*Caulobacter crescentus* is a notable exception [18]). Moreover, even in these cases, the chromosomal origins still show non-random positioning albeit perturbed or altered [19,20,16]. Therefore, it is clear that there are alternative mechanisms for origin positioning yet to be discovered. This is especially the case for bacteria that do not carry Par systems such as *E. coli*, the most widely studied prokaryotic organism, and many of its relatives within the gammaproteobacteria.

Clues can likely be found in a group of DNA maintenance proteins that co-evolved within the gammaproteobacteria, including Dam, SeqA, MukBEF, MatP and MaoP [21,22]. In particular there is increasing evidence that MukBEF, a functional homolog of the widely conserved protein complex Structural Maintenance of Chromosomes (SMC) [23,24], has a role in positioning origins in *E. coli*. Under slow growth conditions, MukBEF forms a small number of dynamic clusters (henceforth foci) located, in close association with *ori* at the middle or quarter positions [25,26] and the splitting and movement of these foci occurs concurrently with the segregation of *ori* to the quarter positions [25,27]. Depletion of MukBEF results in *ori* mis-positioning, which is restored upon repletion [26], suggesting a connection between their positioning. MukBEF recruits the type II topoisomerase Topo IV [27,28], which is required for the timely removal of catenanes from newly replicated sister chromosomes [29]. Support that MukBEF foci positioning *ori* rather than the other way around came from the discovery that depletion of Topo IV results in catenation and mis-positioning of *ori* but not of MukBEF foci [27].

However, up to recently the idea that MukBEF positions *ori* suffered from a fundamental setback. If MukBEF foci position *ori*, what positions MukBEF? Given that foci turnover continuously on a timescale shorter than a minute [30] and that MukBEF binds DNA non-specifically, how does it even form foci? We recently proposed a solution to this conundrum in the form of a self-organising and self-positioning mechanism based on phase fixing a stochastic Turing pattern [31] (see Figure S1a and the methods section for a review). With this model in hand, we are now in a position to test the hypothesis that MukBEF positions chromosomal origins. In particular, we are asking whether the self-organising MukBEF gradient (as described in our previous model) has the correct properties to act as an attracting gradient for *ori*. In particular, it is critical that *ori* are correctly partitioned, i.e. each *ori* is recruited to a *different* MukBEF focus, a non-trivial requirement.

We find that a self-organising MukBEF gradient can indeed accurately reproduce the observed *ori* dynamics, apparent diffusion constant and drift rate. The specific loading of MukBEF at the *ori* introduces a non-trivial interaction between MukBEF foci and *ori* that leads to accurate and stable partitioning as an emergent property of the system. Importantly, the model does not contain any directed force. MukBEF requires energy in the form of ATP to establish a self-organised gradient but it is not pulled to the middle or quarter positions by any active force. Similarly, the attraction of *ori* up the MukBEF gradient is due to energetic considerations and the elastic nature of the chromosome.

Results

Model of *ori* positioning by self-organised MukBEF reproduces mid-cell positioning

As discussed above, multiple lines of evidence point to an interaction between MukBEF foci and *ori* suggest that MukBEF foci position *ori*. How could this interaction be mediated? In *Bacillus subtilis*, SMC, which also colocalises with *ori* [32], is loaded onto the DNA at *parS* sites located mostly in the origin region by the protein ParB [32–34] (see [35] for a review of SMC loading, translocation and function). However, MukBEF and SMC do not show high specific DNA binding [33,36] likely due to their dynamic nature and ability to translocate along the DNA. Thus those studies relied on prior knowledge of ParB-*parS* (similarly for MukBEF unloading by MatP-*matS* [36]). It is therefore conceivable that a loading site/protein exists within the origin region of *E. coli* but has not been identified. We take the presence of such a specific loading site as the starting assumption of the model and we use 'site' without prejudicing the number of sites or the existence of a loading factor.

Given that that MukBEF foci are positioned independently of *ori*, the higher affinity of a loading site suggests that the established MukBEF gradient would provide a potential energy surface for *ori* which is minimised when *ori* are located at peaks in the MukBEF concentration *i.e. ori* would move up MukBEF gradients. From a microscopic point of view, the movement of *ori* is likely due to the elastic nature of DNA [37] allowing it to locally sample the MukBEF gradient and bias its overall direction of movement similar to models of plasmid positioning [38,39] but with the simplification that the protein gradient is already established. Previously, we showed that a specific loading site at a fixed spatial location has an attractive effect on the positioning of self-organised MukBEF foci due to the modified flux differential across foci [31]. Thus the presence of a loading site in the *ori* should lead to an effective mutual attraction between *ori* and MukBEF foci. In order to disentangle the nature of each side of this interaction, we will first examine the movement of *ori* up MukBEF gradients independently of specific loading.

The nature of the attraction of *ori* to its home position has been elegantly characterised by Kuwada *et al*. using time-lapse fluorescence microscopy [9]. They found that the mean *ori* velocity exhibits a linear relationship with the long-axis position, indicative of a diffusing particle experiencing a harmonic (quadratic) potential, or equivalently, a damped spring-like force. Any model of *ori* positioning must be able reproduce this behaviour. However, given the self-organising and fluctuating nature of the MukBEF gradient in our model [31] (which is representative of the *in vivo* behaviour), it was not obvious that it would generate a linear *ori* velocity profile. However, we found that the steady-state distribution of MukBEF (we refer to the slowly-diffusing state that constitute foci [30,31] simply as MukBEF - see methods for details) closely resembles a Gaussian, i.e. the equilibrium distribution for a harmonic potential (Fig. S1b). Thus, MukBEF itself exhibits the same distribution as if it experiencing a damped spring-like force towards mid-cell. As a Gaussian very closely resembles a quadratic around its peak, MukBEF might therefore be able to act, at least in our simulations, as an effective harmonic potential for *ori* and generate the linear velocity profile observed by Kuwada *et al* [9].

Taking initially the case of short cells (2.5 µm), we added a single *ori* into the simulation by treating it is a diffusing particle that can climb the MukBEF gradient (see methods for details). As expected, this led to the *ori* tracking the self-organised MukBEF foci, resulting in mid-cell positioning (Fig. 1a,b and Fig. S1c). Furthermore, as suggested by the steady state MukBEF distribution, we indeed found that simulated *ori* experience a linear restoring velocity (Fig 1c). A quantitative comparison of our simulations with the experimental data from Kuwada et al. [9] was carried out by fitting

the data in the mid-cell region to a theoretical model of diffusion in a harmonic potential, thereby obtaining an apparent diffusion constant and drift rate. We used only cells with a single *ori* focus visible, which, unlike cells with two *ori*, can be grouped together without scaling by simply aligning them according to their mid-cell positions. We found that by adjusting the diffusion and drift input parameters, we were able to obtain excellent agreement with the experimental results (Fig 1c).

However, while promising, these results are not sufficient to suggest that MukBEF can explain the *in vivo* behaviour of *ori*. The challenge arises after the *ori* has been replicated. A true partitioning mechanism must ensure that each replicated *ori* moves to a *different* quarter position. A simple gradient based mechanism cannot, *a priori*, satisfy this requirement as both *ori* could just as easily move towards the same quarter position. Furthermore, the experimental data suggests that once *ori* separate they do not subsequently interchange their positions (cross paths). This ordering is essential during multi-fork replication, where the multiple *ori* of each segregated chromosome must be positioned to the appropriate cell half to avoid guillotining the chromosome upon cell division.

Specific loading leads to stable and accurate partitioning

To examine if the model is capable of accurate and ordered partitioning, we performed simulations with two *ori* in longer cells of 5 µm, in which MukBEF self-organises into, on average, two foci, one at each quarter. The average profile of *ori* positions displayed two peaks centred on the quarter positions. However, looking at individual simulation we found that in approximately half of the simulations both *ori* were near the same quarter position (Fig. 2a), clearly indicating that partitioning was not accurate.

This motivated us to introduce specific loading of MukBEF into the model. As discussed in the previous section, this is likely to lead to a mutual attraction between *ori* and MukBEF. We expected this to increase the association between the two but it was not clear what effect it would have on overall MukBEF-*ori* positioning. Surprisingly, we found that the addition of a specific loading at *ori* results in stable and accurate partitioning (Fig. 2b). A specific loading ratio greater than six (i.e. six times more binding than elsewhere) was sufficient to ensure that one and only one *ori* was positioned to each quarter position and they do not interchange (Fig. 2c,d). Furthermore, when we examined the colocalisation of *ori* with MukBEF foci as has been quantified *in vivo* [27,36], we found that similar levels of specific loading results in very similar cumulative probability distributions of the separation distance (Fig. 2e and S1d).

Additional effects were observed. The positions of both MukBEF and *ori* deviate less from the quarter positions and are almost never at mid-cell (Fig. 2d, Fig. S2a). However this effect was reversed at higher specific loading ratios and we found that six to ten times resulted in the narrowest distribution peaks (Fig. 2f). This did not affect partitioning accuracy or the number of times *ori* crossed paths which remained very high and small respectively (Fig. 2c). However, looking at individual simulations, we observed that the nature of the variance was different. At no or low specific loading, additional (more than two) transient MukBEF foci appear and disappear (Fig. 2a) resulting in a broader average distribution (Fig. 2c); at high specific loading the number of foci was maintained accurately at two (Fig. S2b) and the foci were tightly associated to *ori* but they were both more mobile than at intermediate ratios. We explain this effect and the mechanism behind accurate partitioning below.

Returning to shorter cells with a single *ori*, we measured the effect of specific loading on the apparent diffusion constant and drift rate and found that only the drift rate changes significantly (Fig. S3a,b). However, we are able to choose new

values for the diffusion and drift parameters (at 6x specific loading) such that the agreement with experimental data was recovered (Fig. S3d). Furthermore, the position distribution no longer contained fat tails and was therefore closer to the measured distribution (Fig. S3c).

Ori ensure accurate partitioning by perturbing the balance of fluxes

Let us review the factors determining positioning in the model (Fig. 3a). MukBEF self-organises via a Turing instability into regions of high local density (foci). The position of these foci is determined by the balancing of fluxes originating from a well-mixed (not self-organised) state. With spatially homogeneous conversion from this well-mixed state, the fluxes balance, in the case of a single focus, at the mid-cell position (i). An object (*ori*) that is attracted up the resulting gradient will also be positioned at mid-cell (Fig. 1). However, if the conversion from the well-mixed state is not homogeneous, then the position at which the fluxes balance changes (ii). If higher conversion (loading) occurs at the *ori*, then the mutual attraction between the self-organised MukBEF focus and the *ori* results in strong colocalisation (Fig. 2e) and positioning at mid-cell (iii). The system is analogous to a compound spring with the fixed end at mid-cell (but with a non-trivial relationship between the spring constants and specific loading ratio). At high levels of specific loading, colocalised MukBEF-*ori* becomes less sensitive to the (decreased) incoming flux from other regions, and is therefore less attracted towards mid-cell, resulting in more diffusive behaviour and a broader position distribution (Fig. 2f, S3a).

How do these properties lead to accurate and stable partitioning of *ori*? Suppose both *ori* have been pulled towards the same MukBEF focus (Fig. 3b). Due the flux imbalance, the MukBEF focus without an *ori* will be effectively pulled towards the other one (i) until the fluxes balance (ii). The stochastic nature of *ori* and MukBEF movement mean that at some point one *ori* leaves the influence of its MukBEF focus and becomes associated to the other focus (iii). When this occurs the balance of fluxes is again disturbed and MukBEF-*ori* move as single units towards opposite quarter positions (iv). This is the stable configuration.

Accurate partitioning during growth

The previous two-*ori* simulations were initialised with *ori* at separate quarter positions. When we used random initial positions, we found an identical distribution for the distance between *ori* and the closest MukBEF foci and a similar dependence of the variance (Fig. 2f, dashed lines). However, due to their initial positions both *oris* were sometimes associated to the same MukBEF focus resulting in lower partitioning accuracy (Fig. S2c). We have also previously shown that increased loading can have an inhibitory effect on the growth-induced one-to-two foci MukBEF transition [31].

To further examine this problem, we incorporated exponential growth and stochastic *ori* replication into our simulations. We found that newly duplicated *ori* often remained associated to the same MukBEF focus, resulting in inaccurate partitioning as in the static case (Fig. S4). We also found that specific loading at *ori* delayed the splitting of MukBEF foci, similar to a spatially fixed loading site (Fig. S4b) [31]. However, in the subset of simulation in which partitioning occurred, *ori* were correctly positioned to the quarter positions.

We wondered how the system could be pushed out of the undesirable configuration with both *ori* associated to one MukBEF foci. One possibility has been suggested by polymer simulations. In the concentric shell model [40,41], nucleoid compaction facilitates the onset of segregation by promoting extrusion of newly synthesised DNA to the periphery of the nucleoid. The two *ori* experience the entropic repulsion of two closed loops and separating, initially

rapidly, in opposite directions. This provides a physical mechanism for the capture-extrusion picture of chromosome segregation [42] and is consistent with more recent experimental observations. Duplicated *ori* (and indeed all loci) experience an initially large segregation velocity [10,43] followed by a slower phase in which they approach the quarter positions [9,10].

To mimic this repulsion of replicated *ori*, we incorporated a repulsive force into the simulations (see methods for details). We found that this was able to push the system sufficiently out of the undesirable configuration so that the system reaches the stable correctly partitioned configuration. The MukBEF focus splits, *ori* associate to separate foci and both move towards opposite quarter positions (Fig. 4a-d). The strength of the repulsion and the specific loading rate were chosen to give (at 140 min. simulation time) high (>95%) partitioning accuracy and an average *ori*-separation of half the cell length (see methods for details). Importantly, repulsion between *ori* without specific loading was not sufficient to ensure accurate positioning and partitioning (Fig. S5a,b).

To compare the simulated time-courses to experiment, we synchronized the *ori* trajectories to the time of splitting as has been done for the experimental data [9]. We found excellent agreement of the segregation velocity (the change of the absolute distance between *ori* between time points) and the partitioning accuracy (Fig. 4e,f). We also measured *ori* separation as a function of time and found that it compared favorably but with some disparity, likely due to the broad distribution of cells lengths in the experiments compared to the fixed range used in the simulations (Fig. S5). Overall this good agreement, especially in the absence of any systematic fitting, suggests that *ori* positioning may indeed be explained by their interactions with a self-positioning MukBEF gradient.

Discussion

The chromosomal origin of replication is dynamically positioned within many bacteria. While this can be explained in some bacteria by the presence of ParABS systems, in others, such as *E. coli*, the mechanisms are unknown. In this work, we presented a model for origin positioning in *E. coli* based on self-organising MukBEF, a prokaryotic codensin that is essential for correct chromosome organisation. MukBEF forms foci at the middle and quarter cell positions that show remarkable colocalisation with *ori* in wildtype cells [27,36]. Furthermore, while MukBEF foci are positioned independently of *ori* [27], they are required for *ori* positioning [26]. In previous work [31], we showed how the formation and positioning of MukBEF clusters can be explained by a Turing-type mechanism for self-organisation and positioning.

Here, we extended that model and showed that it could explain origin segregation and positioning and accurately reproduce detailed experimental results. The fundamental assumption of the model is based on the aforementioned relationship with *ori*. We assumed that, like SMC in *B. subtilis*, MukBEF is specifically loaded onto the DNA at specific sites within the *ori*. This could be the case if the *ori* (or proteins bound to it) has a higher affinity for MukBEF than other regions. The first consequence of this assumption is that the self-organised MukBEF gradient can act as a potential energy surface for *ori*, with the result that *ori* climb the MukBEF gradient. This is similar to Brownian ratchet [44,38] and DNA relay models [45] of ParABS-based positioning. However, the situation here is much simpler in that the protein gradient is already established and so *ori* positioning requires no further energy input, similar to the proposed bulk segregation of chromosomes by membrane-based protein gradients [46]. It is also worth noting that MukBEF has been indicated [47–50] to have some ability to multimerize (beyond the dimer of dimer functional unit [30]). This could play a role in the interaction between MukBEF-associated *ori* and the broader MukBEF gradient.

This first property alone was sufficient to explain the observed linear velocity profile in the one-*ori* case but was not able to reproduce accurate partitioning when two *ori* were present (Fig. 2a). However, we found that the higher relative loading rate of MukBEF within *ori* resolved this and achieved better agreement between the distributions of *ori* position (Fig. S3c,d) and *ori*-MukBEF separation distance (Fig. 2e) with the experimental results. Since specific loading perturbs MukBEF foci positioning, pulling them towards the location of the loading site (Fig. 3a(ii)), it means that *ori* and MukBEF are effectively both attracted to each other (Fig. 3a(iii)). Each *ori*-MukBEF behaves as a single unit with *ori* essentially 'piggy-backing' on MukBEF foci, using their intrinsic properties of positioning and mutual repulsion (Fig. 3b).

Finally, we assumed that newly duplicated *ori* experience an effective repulsive force. This is not a new idea but is based on entropic repulsion between polymers [40,41,51]. In our model, this effect pushes the system out of a configuration with both *ori* associated to the same MukBEF clusters (the undesirable configuration), allowing it to fall into the desirable configuration having each *ori* associated to a different quarter-positioned MukBEF cluster. This configuration is stable and does not transition back to the undesirable configuration even in the absence of the repulsive force (Fig. 2). Furthermore, the repulsive force on its own is not sufficient for accurate segregation (Fig. S5). Specific loading at the *ori* is required as this is what leads to the stability of desired configuration (Fig. 3b). The repulsive force is necessary to get the system out of the undesirable state but not for the stability of the desirable state.

Comparing the model to experiment, we found excellent agreement. This is remarkable given the simplistic 1-D reaction-diffusion nature of the model and that we did not perform a systemic fitting of parameters to the experimental data (see methods for details of parameter selection). Furthermore the depth of the comparison is, to our knowledge, beyond what has been done previously for origin segregation in *E. coli*. Hence, we suggest that this approach warrants further consideration and that protein self-organisation may have an unappreciated role in chromosome organisation.

For future work, careful simultaneous measurement of *ori* and MukBEF dynamics would improve parameter estimation and provide ways to directly test the assumptions of the model. In the longer term, combining particle and polymer simulations may provide a deeper understanding of the process. In particular, MukBEF has a major role in chromosome compaction and is responsible for long-range DNA contacts [52]. Like SMC, it may also extrude loops of DNA [52] or be involved in stabilising them [50]. Lastly, recent work has demonstrated that MatP, which binds to *matS* sites in the terminus region, interacts with MukBEF, displacing it from the chromosome [36] and thereby restricting long-range DNA contact in the terminus region [52]. Both effects may help position this region at mid-cell, while simultaneously encouraging the co-localisation of *ori* with MukBEF [36]. Thus, the role of MatP may need to be incorporated into future models. Overall, we envisage that the study of protein self-organisation in the context of chromosome dynamics has a promising future.

Methods

Review of the model

We briefly summarise the underlying model for MukBEF self-organisation [31]. The general model scheme consists of two 'species' u and v interacting in a bounded one-dimensional 'bulk' over a one dimensional surface (Fig. S1a, upper panel). Species u exists in the bulk (the cytosol) with concentration u_c and on the surface (the nucleoid) with concentration u_n. Species v exists only on the surface with concentration v_n. Species v diffuses slower than u. In the context of MukBEF, u_n represents the concentration of the free form of the basic functional complex, the dimer of dimers, while v_n is the concentration of the slower diffusing form of the complex (due to

entrapment of DNA). The former becomes the latter at a basal rate α and cooperatively in a cubic interaction at a rate β, and the latter becomes the former at a rate γ. Both of these species are nucleoid bound. The concentration of the cytosolic state is given by u_c, which converts to and from the bound states with linear rates □ and δ respectively. Figures S1a shows a diagram of the proposed model. See [31] for further details.

The two states u_n and v_n generate the Turing pattern and the kymographs and distributions shown in this work are of v_n (which we simply refer to as MukBEF). The cytosolic state u_c is responsible for the positioning of the pattern and is well-mixed because it interconverts with the Turing species on a sufficiently slow timescale. Note that in general this state is not required to be cytosolic, only well-mixed.

Stochastic Simulations

Stochastic simulations are based on an exact realization of the Reaction Diffusion Master Equation (RDME) using the Gillespie method as described previously [31] but with changes for efficiency and the addition of simulated *ori*. We replaced the binary tree search of the enhanced direct method [53] with a 2D search as proposed by Mauch and Stalzer [54] and switched from 32-bit uniform random numbers (using the Ziggurat method) to 64-bit numbers (using std::mt19937_64) to ensure enough significant digits to accurately sample reactions occurring with very low relative rates (namely, *ori* diffusion). As before, the spatial domain (the long axis of the cell) is divided into discrete compartments, each having a width of $h = 0.1$ µm and between which the species can diffuse. The simulations were extended by the addition of *ori*, treated as an additional diffusing species, as follows.

Diffusion of *ori* subject to the MukBEF gradient: We modelled *ori* simply as diffusing particles. The drift they experience is based on the local MukBEF concentration and we use jump rates (the rate at which *ori* jump between neighbouring compartments) derived by Wang, Peskin and Elston [55]. The forward and backward jump rates from compartment i to $i+1$ and $i-1$ respectively are

$$F_{i,i+1} = \frac{D_{ori}}{h^2}\frac{\alpha_{i,i+1}}{1-e^{-\alpha}}$$

$$B_{i,i-1} = \frac{D_{ori}}{h^2}\frac{\alpha_{i,i-1}}{e^{\alpha}-1}$$

where $\alpha_{i,j} = \frac{\varepsilon(v_j - v_i)}{h}$ is, up to a factor, the difference in the MukBEF concentration between the compartments (v_i is the number of molecules of slowly diffusing MukBEF species in compartment i), D_{ori} is the diffusion constant and ε is the drift parameter, determining the strength of the attraction up the MukBEF gradient. This form for the jump rates, which respects detailed balance, relies on the assumptions that, within individual compartments, the probability density for *ori* is at steady state and the MukBEF gradient is approximately linear. Both of these requirements can be satisfied for sufficiently small compartment widths. However, it is not feasible to decrease the compartment width much below 0.1 µm due to the increased computationally cost but also more fundamentally because of the intrinsic limitation of the RDME for non-linear reactions at short spatial scales [56,57]. Yet, the often sharp MukBEF profile (at a fixed moment in time) suggested that shorter compartment widths might be required. We therefore introduced sub-compartments within every compartment but only for *ori* positions. This approach has previously been applied to stochastic Turing patterns [58] but here we apply it to a 'non-Turing' species (*ori*). Each compartment was divided into an odd number of sub-compartments and the MukBEF concentration was linearly interpolated across sub-compartments. The jumps rates between sub-compartments were then defined as above. Performing simulations for different numbers of sub-compartments, we found that the apparent diffusion constant and drift rate (see below and Fig. 1) stabilised with greater than approximately 5 sub-compartments. The apparent diffusion was approximately 40% higher without sub-compartments. All results presented in this work used 21 sub-compartments as a higher value carried very little computationally cost.

Duplication of *ori*: The timing of *ori* replication was chosen randomly in each simulation by picking a duplication length from a normal distribution with mean 3 µm and coefficient of variation 0.16 (based on the distribution of *ori*-foci splitting length of the data in Kuwada et al. [9]). As we use a fixed growth range (2.5-5 µm) and doubling time (120 min), we truncate the distribution to this

range. This duplication length is then converted to a duplication time via the exponential relationship between cell length and time. The simulation is paused when it reaches this time, the *ori* are duplicated (remaining within the same sub-compartment) and the simulation continued. Note that we do not mimic cohesion of newly replicated stands so that what we refer to in the simulation as *ori* duplication actually more closely corresponds to initial *ori* separation *in vivo*, which occurs 10-15 minutes after replication.

Ori repulsion: As discussed in the text, newly duplicated *ori* are likely to experience, for entropic reasons, a repulsive force between them. We implement this in the model by in the introduction of inverse square force between *ori*. This form of the force was chosen arbitrarily. However, we do not expect the precise nature of the force to affect our results as this force is only required to de-stabilise the undesirable configuration having both duplicated *ori* associated to the same MukBEF focus. It is not required for the existence or stabilisation of the desirable configuration having each *ori* associated to a different MukBEF focus. We assume that we are in the overdamped regime such that the separation velocity due to this force is proportional to the force i.e. $v_s = \frac{\rho}{d^2}$, where d is the distance between *ori*. On the lattice, d is replaced by $d + d_0$, where d_0 is the average distance between two uniformly distributed particles within a sub-compartment in order to the avoid a divergence when both *ori* are in the same sub-compartment. Dividing v_s by the width of a sub-compartment ($h_s = h/21$) defines the propensity function for the reaction that one of the *ori* (randomly chosen) moves one sub-compartment further away from the other *ori*.

Initialisation of simulations: Initial concentrations were set to the homogenous configuration (to the nearest integer). Unless stated otherwise, single *ori* were initially placed at mid-cell, while in simulations starting with two *ori*, they were placed at the quarter positions. Simulations were first run for 30 min to equilibrate and then read out every 1 min (chosen to match the experimental data). For simulations with growth, the simulation was paused after every time-duration that corresponded to growth by one compartment. An additional (empty) compartment was then inserted at a random position and the volume and total number of molecules (via the cytosolic fraction) were increased, maintaining the same overall concentration.

Parameters

All parameters of the core MukBEF model are as previously described, except for the total species concentration, which is increased by 30%. This was done for compatibility of the MukBEF splitting time with the lower range of cell lengths used in this work (2.5 µm – 5 µm), which were chosen to more closely match the range of the experimental data in Kuwada et al. [9]. The remaining (*ori*-related) parameters were chosen by comparison with experimental data as described below.

Apparent *ori* diffusion constant and drift rate

To be able to quantitatively compare the experimental and simulated data, we needed quantitative descriptors of the *ori* dynamics. We compared both data sets to a theoretical model of particle diffusion in a harmonic potential $U = \frac{1}{2} f x^2$ over an infinite 1D domain. Given a particle at position x_0, the probability density that it is at position x at time δt later is [59]

$$p(x, \delta t | x_0) = \sqrt{\frac{f/kT}{2\pi S}} exp\left[-\frac{f/kT}{2S}(x - x_0 e^{-\delta t/\tau})^2\right],$$

where $S = 1 - e^{-2\delta t/\tau}$ and $\tau = \frac{kT}{fD}$. From this, it is straightforward to calculate the expected value and variance of the step-wise velocity $v := \frac{x - x_0}{\delta t}$:

$$E[v] = \frac{e^{\delta t/\tau} - 1}{\delta t} x_0 \approx -\frac{x_0}{\tau}$$
$$Var(v) = \frac{D\tau}{\delta t^2}\left(1 - e^{-2\delta t/\tau}\right) \approx \frac{2D}{\delta t},$$

where the second equality holds for $2\delta t/\tau \ll 1$ (the full expression is used when fitting). Note the expected value of the step-wise velocity depends linearly on position, while the variance is independent of position. This is observed in both experiments and

simulations close in the neighbourhood of the *ori* 'home' position. We therefore use the measured slope of the velocity relationship and its variance to determine an apparent diffusion constant D_a and drift rate $d_a = \frac{1}{\tau} = \frac{fD_a}{kT}$.

Parameter fitting of *ori* related parameters

To search for parameter values for the *ori* diffusion constant (D_{ori}) and the strength of attraction towards MukBEF (ε) that gave agreement between the measured apparent diffusion constants (D_a) and drift rates (d_a), we performed simple parameters sweeps. For the initial fitting (Fig. 1), we chose D_{ori} to be a percentage of the experimentally measured diffusion constant D_a, ranging from 70% to 110% in 5% intervals, while the drift parameter ε was ranged from 0.5 to 2.5 times a nominal value of 0.026 μm (in steps of 0.5). Simulations were performed as described in Figure 1. The combination giving the best agreement and shown in Figure 1 was $D_{ori} = 0.9\, D_a = 5.4 \times 10^{-5}\mu\mathrm{m}^2\mathrm{s}^{-1}$ and $\varepsilon = 0.026$ μm. These values were also used for the simulations shown in Figures 2, S1, S2 and S3a,b.

To produce Figure S3c,d, we performed the same parameter sweep in the presence of 6x specific loading and found that the best agreement was obtained with $D_{ori} = 0.85\, D_a = 5.1 \times 10^{-5}\mu\mathrm{m}^2\mathrm{s}^{-1}$ and $\varepsilon = 2 \times 0.026$ μm (shown in Fig. S3c,d). It should be noted that given the stochastic nature of the simulations, even with 100 runs of 600 min each, there was quite some variability in the data. Furthermore, there appeared to a one parameter family of choices that give acceptable agreement in the diffusion and drift rates. However, they could be distinguished by the kurtosis ('tailedness') of the distribution of *ori* positions and we took this into account. These parameters were used for all subsequent simulations (Fig. 4, S4,S5 and S6).

To find the optimal values for the specific loading ratio and the *ori*-repulsion rate constant (Fig. 4), we used a similar approach. We swept over specific binding ratios of $b = [1,5,10,15,20]$ and repulsion rate constants of $\frac{\rho}{h_s} = [0.005,0.01,0.03,0.05,0.1,0.5]\ \mu\mathrm{m}^2\mathrm{s}^{-1}$. Simulations were performed as described in Figure 4. We examined the fraction of simulations that were correctly positioned and the average *ori*. We found that the combination of $b = 10$ and $\frac{\rho}{h_s} = 0.05\ \mu\mathrm{m}^2\mathrm{s}^{-1}$ was the lowest valued combination that displayed greater than 95% partitioning accuracy and a separation of approximately 50% of the cell length.

To examine the dynamics of new replicated *ori*, we synchronised the simulations to the time of *ori* duplication. We measured the segregation velocity of *ori* (the step-wise rate of change of the absolute distance between *ori*) across the synchronised simulations and found very good agreement with a similar analysis of the experimental data (Fig. 4e). The parameters of the model and the *ori*-duplication length have not been chosen such that, for a given specific loading ratio, the duplication of *ori* is coincident with the length-dependent splitting of the MukBEF focus (due to the computational complexity of such a task). Indeed, *ori* duplication occurs on average at approximately 37 min into the simulation, notable earlier than MukBEF splitting (Fig. 4). Thus, for longer timescale *ori* dynamics, we synchronise the simulations to the last time point at which the *ori* are within 500 nm of each other (results are not very sensitive to the precise value). When we did this we found excellent agreement in the partitioning accuracy and *ori* separation between simulations and experiment (Fig. 4f, S6). With synchronization to *ori* duplication, the *ori* separation profile was sigmoidal with an initial plateau due to both *ori* being in close association with the same and usually only MukBEF focus (as in Figure 4d). Thus, synchronizing as we have done ensures that we assess the dynamics from the time of separation rather than the time of duplication, which is in keeping with the experiments.

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Figure Legends

Figure 1 Ori positioning by a self-organised protein gradient reproduces experimental results

(a) Kymograph from a single simulation showing the number of MukBEF molecules (colour scale) and the position of the *ori* (white line).

(b) and (c) A comparison between the experimental data of Kuwada et al. [9] and the results of simulations in the case of a single *ori*.

(b) Histograms of *ori* position (unscaled) along the long axis of the cell. Zero is the middle position. (c) Mean (top) and variance (bottom) of the step-wise velocity as a function of position. Bars indicate standard error. The linear velocity profile at mid-cell is indicative of diffusion in a harmonic potential ($V(x) = \frac{1}{2} f x^2$). In such a model the variance of the step-wise velocity is independent of position. Thus we obtain the apparent diffusion constant D_a and drift rate $d_a = \frac{f D_a}{kT}$ by fitting to the central region. Bounds are 95% confidence intervals. Simulated data are from 100 independent runs, each of 600 min duration. Experimental data are based on over 16000 data points from 377 cells. Both data sets use 1 min time-intervals. Simulations are from 2.5um cells, whereas experimental data is from a range of cell lengths. See methods for further details and model parameters.

Figure 2 Specific loading of MukBEF at *ori* leads to correct and stable partitioning

(a) Example simulated kymograph showing two *ori* (white lines) diffusing around the same MukBEF peak (colour scale). This occurs approximate 50% of the time.

(b) The addition of 6x specific loading of MukBEF at *ori* positions results in correct partitioning of *ori*. The loading rate in each of the spatial compartments containing *ori* is six times that of the other 48 compartments. The total loading rate is unchanged.

(c) Partitioning accuracy is measured by the fraction of simulations with *ori* in cell halves. Stability is measured by the number of times *ori* cross paths. Both partitioning accuracy and stability increase with specific loading up to approximately 6x. Specific loading ratios are as in (f). Points and bars indicate mean and standard error over independent simulations.

(d) Histograms of *ori* positions for 1x, 6x and 16x specific loading. Positioning is more precise at 6x than with no or 16x specific loading. See Fig. S2a for MukBEF distributions.

(e) The cumulative probability distribution for the separation distance between *ori* and MukBEF peaks. Experimental data (black line) is from Nolivos et al. [36]. The addition of specific loading leads to substantially better agreement. Specific loading ratios are as in (f). See also Fig. S1d.

(f) The variances of individual peaks (obtained by reflecting the data around the mid-position) have a minimum at approximately 6x specific binding. Solid lines are from simulations with *ori* initially at the quarter positions, as for (a)-(e). Dashed lines are from simulations with random initial *ori* positions.

Simulations were performed for a 5 µm domain and two *ori* but otherwise as in Fig. 1.

Figure 3 Schematic of positioning mechanism

(a) With spatially homogeneous loading MukBEF peaks are positioned by regularly positioned by the balance of fluxes (i) [31]. Specific loading purtubs positioning (ii) and combined with the movement of ori up MukBEF gradient leads to robust positioning of both *ori* and MukBEF (iii). They remain in association, moving stochastically but in synch, behaving as if a single entity.

(b) The undesirable configuration of both *ori* associated to the same MukBEF peak is unstable. The lone MukBEF peak is attracted towards the direction of higher flux (i-ii). Both peaks being closer, there is a higher chance that one of the *ori* jumps stochastically into the 'basin of attraction' of the lone peak (iii). The effect of balancing fluxes then causes each MukBEF-*ori* 'complex' to move toward their respective quarter positions (iv), the long-term stable configuration.

Figure 4 Repulsion between newly replicated *ori* promotes the transition to the stable configuration.

(a) and (b) Average kymographs of MukBEF (a) and *ori* position (b) during exponential growth in the presence of a repulsive force between *ori*.

(c) and (d) Two example kymographs from individual simulations (randomly selected) showing number of MukBEF molecules (colour scale) and *ori* position (while lines).

(e) and (f) Segregation velocity (the step-wise rate of change of the absolute distance between *ori*) (e) and partitioning accuracy (f) plotted as function of the time since separation from simulations (blue) and experiment (green). Experimental data is from Kuwada et al. [9] and uses the synchronisation of that work. Shading indicates 95% confidence intervals. See methods for details of the synchronisation of simulated data. The segregation velocity has been corrected for growth. Simulation results are from 450 independent runs and used a doubling time of 120 min.

Figure S1 Properties of MukBEF model and *ori* positioning.

(a) Schematic showing the reactions of the system (see methods). Species *u* exists in the bulk (red) or on the surface (green). Species *v* exists only on the surface (blue). Binding and species interaction are indicated by arrows. Diffusion is not shown.

(b) The average MukBEF profile in the simulations (blue dots) is well approximated by a Gaussian.

(c)Average kymographs from 100 simulations showing the distributions of MukBEF and *ori* positions.

(d) Cumulative probability distribution as in Fig. 2e but for simulation with one *ori*.

Simulations were performed as in Fig. 1.

Figure S2 Additional properties of model in longer cells with two *ori*.

(a) Histograms of MukBEF numbers for 1x, 6x and 16x specific loading. Positioning is more precise at 6x than with no or 16x specific loading.

(b) Fraction of simulation time with two MukBEF foci as a function of the specific loading ratio. Error bars indicate standard deviation.

(c) Partitioning accuracy and stability as in Fig. 2c but using random initial *ori* positions. Data is from the last 100 min of 600 min simulations. Due to both *ori* sometimes being in close initial proximity, the occurrence of undesirable configuration (both *ori* associated to the same MukBEF peak) is higher. Nevertheless the system eventually tends to transition (irreversibly) into the correct configuration, promoted by high specific loading rates. The loading ratios are as in (b).

Figure S3 Additional properties and re-parameterisation of model in short cells with one *ori*.

(a) The variance of the MukBEF and *ori* position distributions for different specific loading ratios for the case of a single *ori*. Simulations as in Fig. 1.

(b) The change in the apparent diffusion constant and drift rate as a function of the specific loading rate.

(c) and (d) A refitting of the simulated data in the presence of 6x specific loading. Otherwise as in Fig. 1b,c. Compare with experimental result in Fig. 1b,c. See methods for further details and model parameters.

Figure S4 The cycle-cycle dependent effect of specific loading.

(a) and (b) Average kymograph of the number of MukBEF molecules and *ori* positions during exponential group with homogeneous loading (a) and 10x specific loading at *ori* (b). Partitioning accuracy is measured by the proportion of simulations having partitioned *ori* at the end of the simulation (140 min).

(c) and (d) Two randomly selected individual simulations from (a) and (b) respectively. The colour scale represents the number of MukBEF molecules; white lines show the positions of *ori*.

Simulation results are from 450 independent runs and used a doubling time of 120 min.

Figure S5 Repulsion between *ori* is not sufficient for correct segregation and positioning.

(a) Average kymograph of the number of MukBEF molecules and *ori* positions during exponential group with no specific loading but

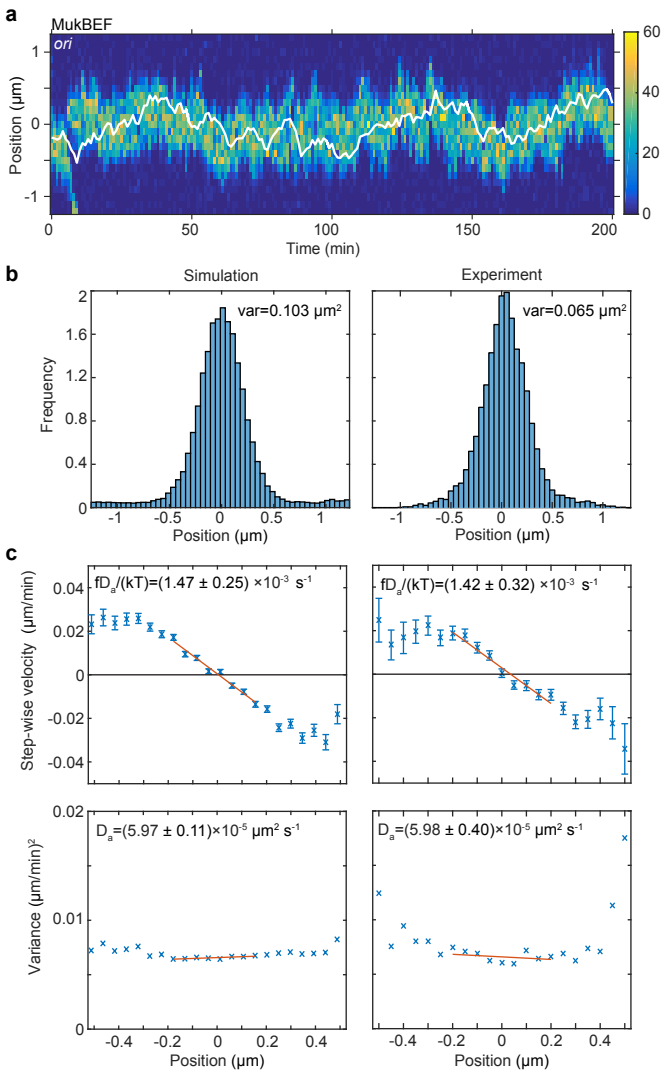
with repulsion between *ori*. Partitioning accuracy is measured by the proportion of simulations having partitioned *ori* at the end of the simulation (140 min).

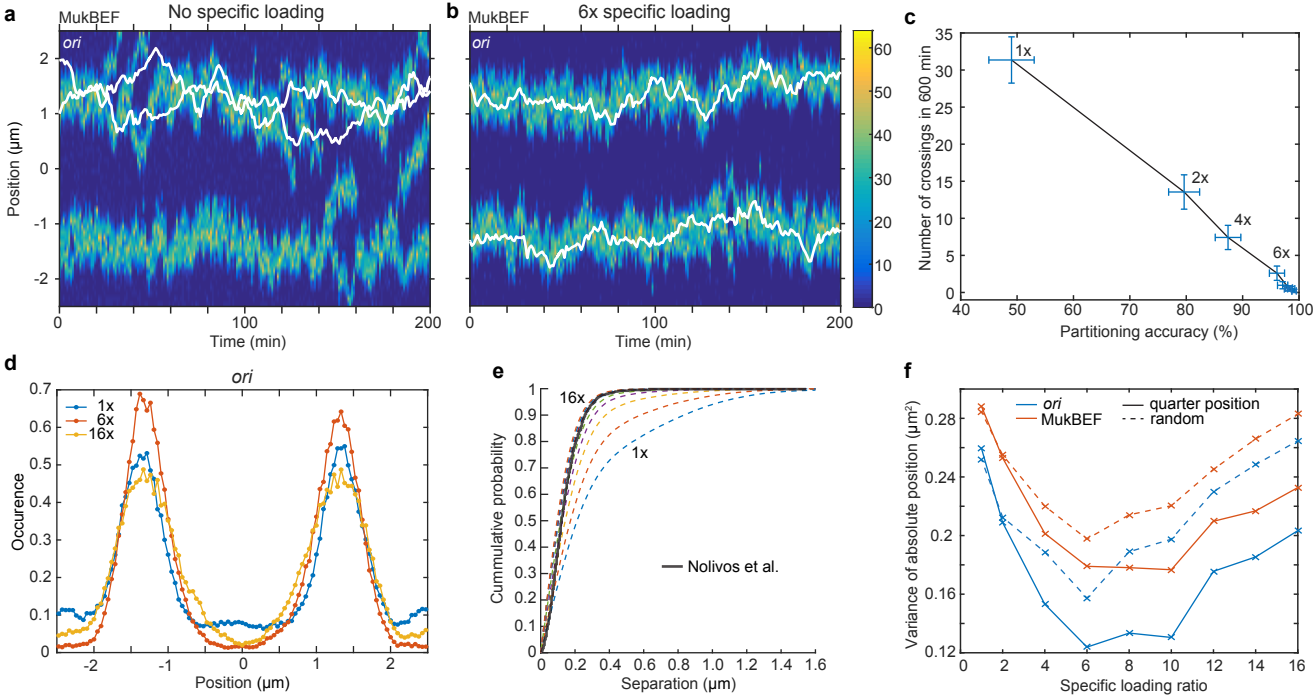
(b) Two representative individual simulations from (a). The colour scale represents the number of MukBEF molecules; white lines show the positions of *ori*.

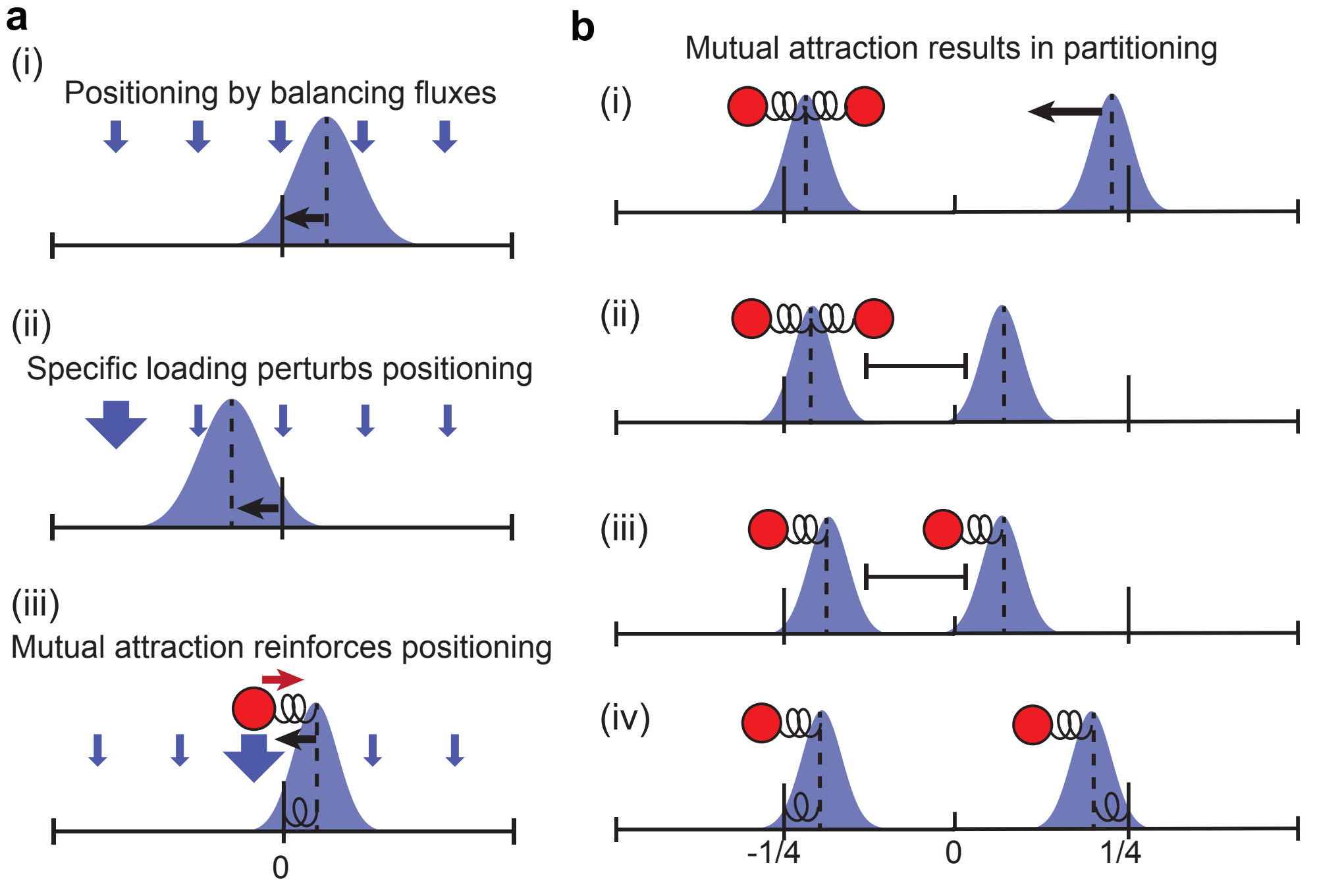
Simulation results are from 450 independent runs and used a doubling time of 120 min. See Fig. 4 for the effect of repulsion in the presence of specific loading. The strength of the repulsive force is the same as in Fig. 4.

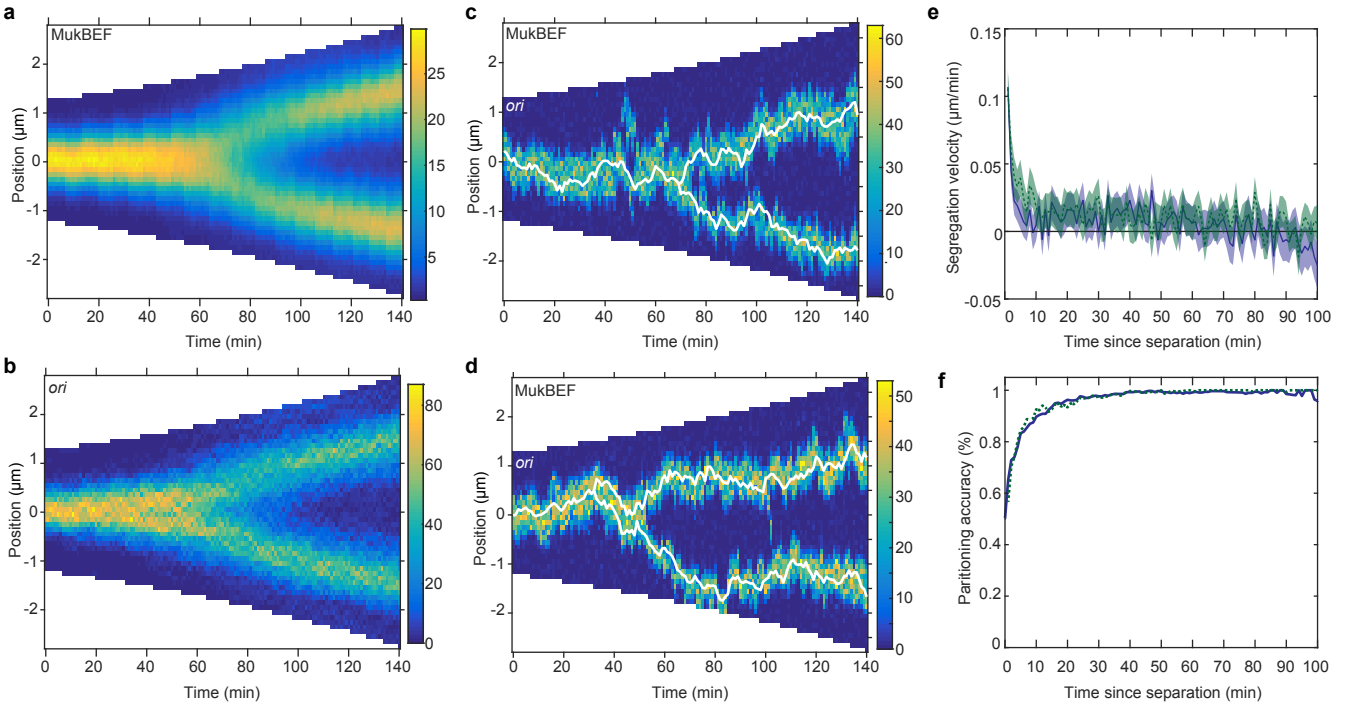
Figure S6 The *ori* separation distance is in qualitative agreement with experimental results.

The *ori* separation distance relative to cell length plotted as function of the time since separation. Experimental curve obtained using the data of Kuwada et al. [9]. Simulated data is as shown in Fig. 4. Individual simulations were synchronised to the last time point for which the *ori* are within 0.5 µm of each other (as for partitioning accuracy in Fig. 4f). See methods for details. Shading indicates 95% confidence intervals.


a
MukBEF
ori
Position (μm)
Time (min)
b
Simulation
Experiment
var=0.103 μm²
var=0.065 μm²
Frequency
Position (μm)
c
fDa/(kT)=(1.47 ± 0.25) ×10⁻³ s⁻¹
fDa/(kT)=(1.42 ± 0.32) ×10⁻³ s⁻¹
Step-wise velocity (μm/min)
Da=(5.97 ± 0.11)×10⁻⁵ μm² s⁻¹
Da=(5.98 ± 0.40)×10⁻⁵ μm² s⁻¹
Variance (μm/min)²
Position (μm)


a
MukBEF
No specific loading
ori
Position (µm)
Time (min)
b
MukBEF
6x specific loading
ori
Time (min)
c
Number of crossings in 600 min
Partitioning accuracy (%)
1x
2x
4x
6x
d
ori
1x
6x
16x
Occurence
Position (µm)
e
16x
1x
Nolivos et al.
Cummulative probability
Separation (µm)
f
ori
MukBEF
quarter position
random
Variance of absolute position (µm²)
Specific loading ratio


a
(i)
Positioning by balancing fluxes
(ii)
Specific loading perturbs positioning
(iii)
Mutual attraction reinforces positioning
0
b
Mutual attraction results in partitioning
(i)
(ii)
(iii)
(iv)
-1/4
0
1/4


a
MukBEF
Position (μm)
Time (min)
b
ori
Position (μm)
Time (min)
c
MukBEF
ori
Position (μm)
Time (min)
d
MukBEF
ori
Position (μm)
Time (min)
e
Segregation velocity (μm/min)
Time since separation (min)
f
Paritioning accuracy (%)
Time since separation (min)