Self-organization and positioning of bacterial protein clusters

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Many cellular processes require proteins to be precisely positioned within the cell. In some cases this can be attributed to passive mechanisms such as recruitment by other proteins in the cell or by exploiting the curvature of the membrane. However, in bacteria, active self-positioning is likely to play a role in multiple processes, including the positioning of the future site of cell division and cytoplasmic protein clusters. How can such dynamic clusters be formed and positioned? Here, we present a model for the self-organization and positioning of dynamic protein clusters into regularly repeating patterns based on a phase-locked Turing pattern. A single peak in the concentration is always positioned at the midpoint of the model cell, and two peaks are positioned at the midpoint of each half. Furthermore, domain growth results in peak splitting and pattern doubling. We argue that the model may explain the regular positioning of the highly conserved structural maintenance of chromosomes complexes on the bacterial nucleoid and that it provides an attractive mechanism for the self-positioning of dynamic protein clusters in other systems.

Pattern formation in multi-cellular biology is often investigated using a reaction–diffusion mechanism in which a diffusion-driven instability results in spontaneous patterning. First applied to morphogenesis by Turing, this has since become established in many systems in developmental biology such as skin pigmentation, hair patterning and the positioning of digit primordia. Typical requirements for Turing pattern formation are differing diffusion rates or, in certain cases, reaction rates and a cooperative higher-order interaction.

However, spatial organization is also essential within cells, where many cellular processes such as cell division, DNA segregation, cell polarity and motility require the positioning of proteins to specific locations within the cell. In bacteria, this is often achieved by passive mechanisms such as recruitment or repulsion by existing landmark proteins or membrane curvature sensing. Truly active mechanisms that do not rely on a pre-existing marker or gradient have largely been confined to DNA segregation. However, several potential systems for determining the future division site have also been discovered. How can such protein complexes, which can be very dynamic and exhibit rapid turnover, be positioned?

Geometry sensing can occur due to a difference in the local bulk volume and unequal membrane affinities. However, the resulting pattern labels only geometrically distinct regions and cannot produce a repeating regular pattern along the length of a rod-shaped cell. Turing-type mechanisms, on the other hand, have not been applied very much in the intra-cellular context (a notable exception is yeast polarity). This is probably due to the sensitivity of the mechanism to initial conditions and parameters—the ‘fine-tuning’ problem of mode selection and the ‘robustness problem’ of maintaining a given pattern. This is probably due to the sensitivity of the mechanism to initial conditions and parameters—the ‘fine-tuning’ problem of mode selection and the ‘robustness problem’ of maintaining a given pattern. The pattern, when it exists, does not generally have a fixed pre-determined phase. Note that while classically referring only to static patterns (the focus of this work), the Turing mechanism can also induce oscillatory patterns, which have been studied extensively in the context of the Min system.

Here, we present a model for the self-organization and positioning of dynamic protein complexes using a phase-locked Turing pattern. The model produces a regular repeating pattern that is insensitive to initial conditions and has a fixed phase. Nucleoid–cytosol exchange essentially selects and phase-locks the Turing pattern even though many modes are linearly unstable, overcoming the drawbacks mentioned above. In short cells the mechanism results in a single focus at mid-cell, while in longer cells a focus is positioned at each quarter position and so on with increasing length. Furthermore, the system exhibits pattern doubling due to domain growth and can be controlled and made more precise by inhomogeneous binding. The model is motivated by the self-positioning of the structural maintenance of chromosomes (SMC) protein complexes on the bacterial nucleoid and is generally applicable to other systems.

A model of MukBEF cluster formation and positioning

Ubiquitous in all domains of life, SMC protein complexes are condensins, required for correct chromosome condensation, organization and segregation. MukBEF, the Escherichia coli SMC, consists of a dimer of MukB, joined at a hinge domain and at an ATP-binding head domain to form a loop capable of entrapping DNA, together with the small accessory proteins MukEF. Binding of MukB to DNA is highly cooperative. Both in vivo and in vitro studies suggest that ATP serves as a DNA-binding switch: ATP binding promotes loading onto DNA, while ATP hydrolysis stimulates detachment. Studies have shown that MukBEF forms clusters in the middle of the nucleoid in short E. coli cells and this localization changes to the two quarter positions in longer cells. However, the mechanism of this positioning is unknown. A live-cell study showed that the fluorescent foci consist of relatively immobile complexes, composed of 8–10 MukBEF dimer of dimers that were suggested to have entrapped multiple DNA strands, and another pool that diffuses more rapidly.

How are MukBEF clusters formed and positioned? Non-specific DNA binding and the requirement of ATP hydrolysis suggest a directed energy-dependent mechanism, rather than simple recruitment. We hypothesized that the system could be described by Turing pattern formation. This was motivated by two properties

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The general model scheme consists of two ‘species’ \( u \) and \( v \) interacting in a bounded one-dimensional ‘bulk’ over a one-dimensional surface (Fig. 1a, upper panel). Species \( u \) exists in the bulk (the cytosol) with concentration \( u_c \), and on the surface (the nucleoid) with concentration \( u_m \). Species \( v \) exists only on the surface with concentration \( v_c \). Species \( v \) diffuses slower than \( u \). In the context of MukBEF, \( u_m \) represents the concentration of the free form of the basic functional complex, the dimer of dimers, while \( v_m \) is the concentration of the slower diffusing form (due to entrapment of DNA) of the complex and \( u_c \) is the concentration of the cytosolic described above: two populations with differing diffusion constants; and the presence of cooperative (higher-order) reactions.

The partial differential equations describing the system.

\[
\begin{align*}
\frac{\partial u_m}{\partial t} &= D_u \frac{\partial^2 u_m}{\partial x^2} - \alpha u_m + \beta u_m v_m^2 - \gamma v_m + \epsilon u_c - \delta u_m \\
\frac{\partial v_m}{\partial t} &= D_v \frac{\partial^2 v_m}{\partial x^2} + \alpha u_m + \beta u_m v_m^2 - \gamma v_m - \delta v_m \\
\frac{\partial u_c}{\partial t} &= D_u \frac{\partial^2 u_c}{\partial x^2} - \epsilon u_c + \delta u_m + \delta' v_m \\
\frac{\partial u_c}{\partial x} &= 0, \quad \frac{\partial v_m}{\partial x} = 0 \text{ on } x = 0, L.
\end{align*}
\]

Figure 1 | A self-positioning Turing pattern. a. Upper panel: schematic showing the reactions of the system. Species \( u \) (green) exists in the bulk (lighter colour) or on the surface (darker colour). Species \( v \) exists only on the surface. Binding and species interaction are indicated by arrows. Diffusion is not shown. Lower panel: schematic showing the flux-balance mechanism. The thinner arrows represent binding or diffusion. The thick arrow indicates the direction of foci movement. b. The partial differential equations describing the system. \( L \) is the domain (system) length. c. Kymographs of \( v_m \) from a solution of the equations in b over 2 and 4 \( \mu \)m domains. d. Top: the average distribution of \( u_m \) (green), \( v_m \) (blue) and \( u_c \) (red) after 30 min for 2 \( \mu \)m and 4 \( \mu \)m domains over 200 different random initial conditions. The contribution of the boundary modes are visible. Bottom: histograms of the dominant non-zero mode at the end of each simulation. Even modes with negative amplitude correspond to regularly positioning interior peaks (red bars). See Supplementary Fig. 2b for the case of a 3 \( \mu \)m domain. e. Example kymographs of \( v_m \) from stochastic simulations over 3 \( \mu \)m and 6 \( \mu \)m domains. See Supplementary Fig. 5 for examples of single long-time simulations. f. The stochastic system was run 200 times as in e and the distributions (top) of molecules of \( v_m \) (green) and \( v_m \) (blue) were obtained by averaging over the last 10 min of the simulations. Boundary modes are not visible above stochastic effects. Bottom: histograms of the dominant mode of each simulation.
state. Figure 1a,b shows a diagram of the model and the respective equations. See the Methods for further details.

**A self-positioning Turing pattern**

The system was found to self-organize with localized high concentrations of species $v$ (Fig. 1c). Interestingly, we observed that the phase of the pattern is nearly always fixed. For our choice of parameters, there was generally a single peak in $v_{\text{m}}$ positioned at the midpoint (mode 2), see Methods) on a 2 $\mu$m domain, whereas there were peaks at each quarter position (mode 4$^-$) on a 4 $\mu$m domain (Fig. 1c,d). This is in stark contrast to the prediction from linear stability analysis (Supplementary Fig. 1b) that many modes, both even and odd, are driven spatially unstable by diffusion, with modes $n = 4$ and $n = 8$ respectively having the greatest growth rates (see Methods).

For a small set of initial conditions, foci stabilized on the boundary (Fig. 1d and Supplementary Fig. 2a). Otherwise, however, the phase of the pattern was fixed as above. Furthermore, this was the case even for initial configurations far from the homogeneous steady state. Note that the Turing mechanism employed here does not produce static foci, rather the peaks constitute regions of high molecular density, with molecules free to diffuse in and out of the region—the model contains no oligomerization beyond that of the basic functional complexes. We found similar behaviour in three dimensions (Supplementary Fig. 3).

To probe the nature of the effect, we considered the system without the bulk form of $u$—both $u$ and $v$ remain confined to the surface. Similar patterns form but they are not self-positioned. Foci do not move even over very long timescales (Supplementary Fig. 4a,b), although they could, as in the full system, disperse before the final pattern stabilized. Stable patterns were dominated by a much broader distribution of modes than in the full system (Supplementary Fig. 4c).

These results demonstrate that un-/binding of molecules is necessary for dynamic self-positioning of foci. We explain this effect by a flux-balance argument (Fig. 1a, lower panel, Supplementary Text). The flux of $u$ molecules binding from the bulk to any particular region is proportional to the region area. If a high-density focus of $v$ molecules has formed off-centre, the flux of $u$ molecules reaching the focus from each side is proportional to the area (length) of the domain on each side. The difference in flux from either side results in a locally higher reaction rate from $u$ to $v$ on one side than the other, which, for a single focus, causes it to move to midcell, where the fluxes on either side balance. On a larger domain with multiple foci, the same argument leads to regular positioning. This very general argument has been previously made for regular plasmid positioning and, albeit implicitly, for the self-assembly and positioning of the lateral chemotactic clusters in *E. coli* [2], which however are both essentially static objects. Here, however, nucleoid–cytosol exchange, in combination with a Turing instability, results in the self-organization and positioning of highly dynamic protein clusters. Importantly, unlike the plasmid case, the positioning is done by the same proteins that constitute the object, rather than a separate positioning system.

**Stochastic effects increase robustness of positioning**

Biological systems are subject to noisy environments and perturbing stochastic effects and this is especially true for proteins with small cellular concentrations. Recent estimates for the number of MukBEF molecules in the cell are in the range of 200–500 molecules [25,26]. We therefore developed stochastic simulations to investigate the effect of noise on the positioning mechanism.

We found that foci fluctuated around the middle position or quarter positions (Fig. 1e). The distributions obtained over long-time simulations showed clear maxima at these positions (Supplementary Fig. 5), as did averaging many independent simulations (Supplementary Fig. 2c). Foci occasionally localized on the domain boundaries (Fig. 1e,f) but such patterns were not stable and after a short time the system returned to the correct position (Fig. 1e and Supplementary Fig. 5). As in the deterministic case, positioning is lost in the absence of exchange with the bulk (Supplementary Fig. 4d,e). The flux-balance mechanism evidently biases selection of both the desired regularly positioned symmetric modes and the undesirable boundary modes, while stochastic effects destabilize the latter (see Methods and Supplementary Text). Furthermore, pattern formation and positioning occurred over a relatively wide parameter range, especially in the stochastic case (Supplementary Fig. 1c and Methods). These results demonstrate the robustness of the patterning and its independence on initial conditions.

**Peak splitting is induced by growth**

Timely splitting and repositioning is often central to the function of positioning mechanisms in growing cells. We therefore investigated the effect of an exponentially growing domain on foci positioning. We observed that a peak-splitting event occurs, in which the mode 2$^-$ solution abruptly splits into the mode 4$^-$ solution (Fig. 2a). Treating the domain length as a bifurcation parameter, we found the critical domain length to be $L_{\text{crit}} = 5.45 \mu$m (Fig. 2b). Interestingly, a shrinking domain causes the opposite bifurcation but at a lower critical length of 2.71 $\mu$m, indicating a type of hysteresis (Fig. 2b and Supplementary Fig. 6a). This bifurcation does not occur via peak merging, rather one of the two peaks disperses, while the other moves to the mid-domain position. We found that the critical length $L_{\text{crit}}$ increases with both on-nucleoid diffusion rates. However, increasing the cytosolic diffusion constant has no effect since the cytosol is already well mixed on the timescale of protein un-/binding (Supplementary Text).

Turning to stochastic simulations, we found much the same behaviour with one focus at mid-domain rapidly becoming two foci at the quarter positions (Fig. 2c). Peak splitting occurred earlier than in the deterministic simulations and generally between 40 and 80 min into the simulation with half of the simulations having split by 60 min/4.1 $\mu$m (Fig. 2d and Supplementary Fig. 6b), whereas the critical length is only reached at 112 min. It therefore appears that the bifurcation is analogous to a saddle-mode bifurcation in that the basin of attraction of the stable fixed point shrinks as the bifurcation is approached. Stochastic fluctuations then allow the system to jump to the mode 4$^-$ branch before the bifurcation occurs.

Similar to what has been shown before [15], we found that extended exponential domain growth leads to robust pattern doubling of Turing patterns in the deterministic case (Supplementary Fig. 7). On the other hand, pattern doubling has been discovered not to be robust in the stochastic case [13]. Indeed, we observed that foci are regularly positioned but do not split simultaneously like in the deterministic case (Supplementary Fig. 8a). However, we found that the average number of foci shows a clear linear relationship with the domain length with 1 additional focus every 3 $\mu$m (Supplementary Fig. 8b). Therefore, while foci do not split synchronously in individual simulations, the average growth behaviour exhibits pattern doubling. Similar regular positioning of foci has been observed for SgsA within long aerial hyphae in *Streptomyces coelicolor* [13].

To qualitatively compare these results on peak splitting with the biological system, we imaged a strain carrying a MukB-GFP fluorescent fusion [15]. MukB forms clusters at the mid- or quarter-cell positions and these clusters can be composed of one to two closely spaced foci, which interconvert reversibly and dynamically within a time frame of less than 5 min (ref. 32), reminiscent of what we observe in our individual simulations (Figs 1e and 2c and Supplementary Figs 5 and 8). We performed an averaging over many stochastic simulations (Fig. 3a) and compared to an averaged demograph of MukB-GFP intensity. We found a very similar pattern in both with a clear relationship between the cell length and...
found that the nucleoid starts to become bi-lobed only after the
performed a demographic analysis as for MukB-GFP (Fig. 3d). We
splitting? To address this, we stained the nucleoid in live cells and
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separated foci to simply forming and positioning multiple single
(Fig. 3c and Supplementary Fig. 6b). Chromosome segregation
splitting earlier and more synchronously than domain growth alone
(cytosol). Interestingly, we found this was able to induce focus
into two compartments, while still allowing exchange via the bulk
segregation in our simulations by splitting the surface (nucleoid)
to be severely restricted with the majority of protein exchange
results are qualitatively consistent with the growth-induced splitting
in our model.

Nucleoid compartmentalization can enhance peak splitting
Nucleoid-bound proteins are inherently affected by chromosome
segregation: diffusion between the two nucleoid lobes is likely to be severely restricted with the majority of protein exchange occurring via the cytosol. We therefore mimicked chromosome segregation in our simulations by splitting the surface (nucleoid) into two compartments, while still allowing exchange via the bulk (cytosol). Interestingly, we found this was able to induce focus splitting earlier and more synchronously than domain growth alone (Fig. 3c and Supplementary Fig. 6b). Chromosome segregation essentially reduces the problem of forming and positioning multiple separated foci to simply forming and positioning multiple single foci individually and may therefore contribute to the robustness of systems such as that of SsgA.

Does nucleoid segregation play a role in MukBEF clusters splitting? To address this, we stained the nucleoid in live cells and performed a demographic analysis as for MukB-GFP (Fig. 3d). We found that the nucleoid starts to become bi-lobed only after the MukB-GFP clusters splitting. Importantly, the model parameters are the same as in the static case and have not been specifically chosen to obtain agreement with the experimental result. While not directly proving that splitting of MukB-GFP clusters is due to growth, these results are qualitatively consistent with the growth-induced splitting in our model.

Inhomogeneous binding modulates positioning
In the model presented thus far, we have taken un-/binding of proteins to be uniform. However, many nucleoid-associated proteins exhibit some amount of specific DNA binding. Both E. coli MukBEF and Bacillus subtilis SMC do not exhibit sequence-specific DNA binding\(^\text{36,37}\). However, the presence of other proteins cause the latter to be specifically loaded onto the DNA at sites in the ori region\(^\text{36,38}\), while the former is specifically unloaded from the DNA at sites in the ter region\(^\text{26}\). In our model, we found that a specific binding site at a quarter position attracted the focus away from mid-domain (Supplementary

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Inhomogeneous binding can enhance peak splitting

We next asked what effect inhomogeneous un-/binding would have in the presence of growth. We found that maintaining a single specific binding site at the mid-domain position resulted in more precise positioning and a higher local concentration of molecules while a single focus was present and the opposite while there were two foci (compare Fig. 4a,d with Fig. 3a and Supplementary Fig. 6b). Additionally, focus splitting was greatly delayed. Interestingly, maintaining a higher-affinity site at each quarter position resulted in the opposite effect with more precise positioning while there were two foci and less while there was a single focus and earlier splitting (Fig. 4b,e). Finally, we considered a specific unbinding site maintained at the mid-domain position. We found a delay in focus splitting and mis-positioning when a single focus was present (Supplementary Fig. 10c,d). The focus generally stabilized on one side of the mid-position, resulting in a bi-modal distribution on average.

Every chromosomal site is duplicated and positioned during the cell cycle. For example, the ori regions in slow-growing *E. coli* cells are positioned at mid-cell before being replicated and migrating rapidly to the quarter positions. We mimicked such behaviour by a discrete duplication and repositioning event. We found that this was able to accurately set the time of splitting and repositioning to within a 10 min window (Fig. 4c,f). Furthermore, as the ‘default’ positions of the foci matched the locations of these sites, the localization was more precise throughout the entire simulation than in the case of homogeneous binding.

These results indicate that while specific binding (unbinding) is not necessary for the self-positioning mechanism described here, it can override the inherent positioning of the mechanism by having an attractive (repulsive) effect. When the location of a site-specific binding site coincides with the site ‘preferred’ by the mechanism then this additional positive influence results in more precise positioning. When this is combined with duplication and segregation of the specific binding site, foci splitting is made synchronously with this event.

**MukBEF localization as a self-positioning Turing pattern**

The *E. coli* SMC complex, MukBEF, is required for efficient chromosome segregation and organization. Through an unknown mechanism, it forms nucleoid-bound clusters at mid-cell or, in longer cells, at the quarter positions. The first major result of this work is an explanation for this behaviour based on the current understanding of the system and using the measured diffusion...
and binding rates. We found that the system is capable of self-organization, forming high-density regions (foci) spontaneously and in the absence of stable complex–complex interactions. Furthermore, the exchange of molecules between the nucleoid and the cytosol results, via balancing fluxes, in foci positioning, placing a single focus at mid-cell or, in longer cells, a focus at each quarter position. We found un-intuitively that stochasticity improved the accuracy of positioning in that sometimes foci stabilized on the boundary in the deterministic case.

Two observations further support our model of dynamic self-positioning. Firstly, positioning is perturbed in a strain with a mutant MukBEF that can bind but only weakly hydrolyse ATP. Since ATP release results in unbinding from DNA, this mutant is described in our model by blocking unbinding from DNA, which also led to mis-positioned foci (Supplementary Fig. 4). Secondly, repletion of the MukF subunit in non-replicating cells actually associate with MatP-ori, The corresponding mean number of foci (blue). For comparison, the homogeneous case from Fig. 3a and Supplementary Fig. 6b is shown in red. The shaded region represents one standard deviation. The specific binding rate is 15 times that of the other positions. This intriguing possibility is now bolstered by the self-positioning mechanism that we have presented here.

**Self-organization and positioning of protein clusters**

Protein clusters can be very dynamic with rapid turnover and yet be regularly positioned within the cell. We explain this behaviour using a Turing-type reaction–diffusion mechanism for self-organization. Positioning is due to surface-bulk (nucleoid–cytosol) exchange leading to a disparity in fluxes on the surface. A similar flux-balance argument has been proposed for the positioning of (non-dynamic) plasmids by the ParABS system. Here, however the foci (protein clusters) are dynamic objects that self-organize and can position themselves independently without relying on any external positioning proteins. Furthermore, while flux balance can provide the spatial information to position plasmids, the actual molecular mechanism for plasmid movement is unknown, with several competing models. Here, however, the self-organization and positioning are self-contained, requiring only the basic reactions of the system (Fig. 1a).

The key ingredients of the model are the following: a protein with cooperative self-interactions that exists in two nucleoid-bound populations with differing diffusion rates and which exchange with a well-mixed cytosolic population. Additionally, energy expenditure (for example, ATP hydrolysis) is required for self-organization and the positioning mechanism requires that this be linked to unbinding from the nucleoid. Importantly, oligomerization is not a requirement. Given the generality of the model, we expect it to be applicable to other systems with these properties, such as the cytoplasmic chemotactic clusters in Rhodobacter sphaeroides and the PomZ cluster of Myxococcus xanthus.

**A phase-locked Turing pattern**

Finally, from a mathematical viewpoint, this work presents a reaction–diffusion system that results in a specific pattern with a fixed phase, is not sensitive to initial conditions and is robust.
to perturbations in parameters. Phase fixing has typically been achieved by tuning the model parameters such that a single mode is driven unstable by diffusion and hence dominates the final pattern, or by using fixed or mixed boundary conditions. Here, we showed that stochastic effects cause the ‘undesirable’ boundary patterns to be destabilized, ensuring that which pattern is formed is independent of the initial perturbation, that is, the stochastic pattern consists solely, on average, of a parameter and domain-size dependent number of interior regularly positioned peaks. This result will contribute to the application of the Turing mechanism in explaining biological pattern formation.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

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**Author contributions**

S.M.M. initiated the work, conceived the model and designed and performed simulations and experiments. V.S. contributed to experiment design. Both authors discussed the results and implications. S.M.M. wrote the initial draft of the manuscript. Both authors edited subsequent and final versions of the manuscript.

**Additional information**

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Correspondence and requests for materials should be addressed to S.M.M.

**Competing financial interests**

The authors declare no competing financial interests.
Methods

Model. We developed a model of protein self-organization based on the biological properties of MukBEF (summarized in the Supplementary Text). The concentration functional unit, the free dimer of dimers, is represented by \( u_n \), while the concentration of slower diffusing dimer of dimers is given by \( v_n \). Both of these species are nucleoid-bound. The slower diffusion is believed to be due to entrapment of multiple DNA strands\(^{52}\). The former becomes the latter at a basal rate \( \alpha \) and cooperatively at a rate \( \beta \), and the latter becomes the former at a rate \( \gamma \). That is, slowly diffusing multimerized cooperatively recruit additional quickly diffusing subunits. We envisage the following mechanism for this recruitment: Transient higher-order multimerization, which has been observed in vitro\(^{53,54}\), may act to anchor the quickly diffusing complex, allowing time for each of the two constituent dimers to engulf a DNA strand each, thus greatly reducing the mobility of the complex. This state then defines the species \( v_n \). Such multimerization would have to be sufficiently transient (not much longer than the timescale of unbinding) so that complexes are not locked in place and therefore not able to actively position. We describe this interaction by the \( u_n v_n^2 \) term, postulating that two \( v_n \) complexes are required to transiently anchor one \( u_n \) complex. While other possibilities, such as those containing Michaelis–Menten-type factors, also work, this term has the benefit of being well studied in context of Turing pattern formation, for example, as part of the Schnakenberg, Gray–Scott and Brusselator models (see ref. 51 for a review).

The concentration of the cytosolic (ATP-unbound) state is given by \( u_n \). We model the ATP diffusion terms of the cytosolic state to the DNA-bound dimer of dimers (\( u_n \)) simply by a single linear rate \( \epsilon \), since it is unknown whether the intermediate ATP-bound complex dimerizes before or after binding DNA. Similarly, the hydrolysis and unbinding of the nucleoid-bound species are represented by single linear rates \( \delta \) and \( \delta' \), respectively. The concentrations are in terms of the MukE subunit, the stoichiometry of which within complexes does not change with the above definitions and hence the sum of three concentrations is conserved.

For the choice of parameters below the system has only one fixed point, found numerically to be stable (Supplementary Fig. 1a). Pattern formation can therefore occur starting from any initial conditions given a parameter set that results in a Turing instability around this single fixed point, that is, the system will not be attracted to some other non-pattern-forming fixed point.

The do not provide a full analytical treatment of the stability of the system or of the conditions sufficient for Turing pattern formation due to the complexity of the solution (at least without setting parameters to trivial values). Determining the fixed points of the system requires solving a cubic equation, having 1 or 3 positive roots (no negative roots) as can be seen in Supplementary Fig. 1a. However, for the special case of \( \epsilon = 0 \), there is a fixed point at \( v_n = 0 \), which is always stable and, if they exist, the fixed point with the highest value of \( v_n \) is also stable while the intermediate fixed point is a saddle point. However, as discussed above we take a non-zero value of \( \epsilon \) to ensure that there is only one fixed point.

For reasons of computational complexity, we largely restrict ourselves to one spatial dimension. This is justified since E. coli is a rod-shaped bacterium and by the large size of fluorescent foci in comparison with the nucleoid\(^{51} \).

Deterministic simulations. The system of partial differential equations was solved in Matlab using the pdepe solver. The system was integrated over a mesh with a spacing of 0.05 \( \mu \)m. Initial conditions were taken to be a random perturbation about the homogeneous steady state (drawn from a normal distribution with standard deviation of 10%). For simulations involving growth, the Lagrangian formalism was used for computational efficiency and the solutions mapped back to the Eulerian formalism. The three-dimensional solution was obtained using a custom-written code based on the method of finite differences.

Stochastic simulations. Exact stochastic (Gillespie) simulations\(^{52} \) were implemented in C++ and based on the enhanced direct method (EDM)\(^{53} \). We also combined the sorted linear ordering of propensities of the optimized direct method\(^{54} \) with the binary tree search of the EDM: the propensities were sorted into reaction types (Diffusion of species 1, Diffusion of species 2, Binding of species 1 and so on) and stored with their partial sums in individual binary trees. The roots of these trees were then sorted in descending order to be searched linearly as in the optimized direct method, followed by a binary search up the tree as in the EDM. We used the zigzag method for generating random variables. In the stochastic simulations the bulk is assumed to be well mixed. This is justified by the much slower timescale of binding/unbinding compared with that of diffusion, which results in a homogenous bulk concentration of species 1 and 2, independence of the one to two peak transition on higher cytosolic diffusion rates (Fig. 2b, inset). The spatial aspect is dealt with by dividing the domain into compartments, each having a width of 0.1 \( \mu \)m (apart from the simulations in Supplementary Fig. 8, which used 0.15 \( \mu \)m) and between which the species can diffuse. Initial concentrations were set to the integer homogeneous configuration closest to the deterministic model. The stochastic system was simulated using the same fundamental parameters as for the deterministic case (appropriately converted from units of nanomolar and micrometres to number of molecules and compartment length respectively). The system state was read out every 5 or 10 \( \mu \)s. For simulations with growth, the simulation was paused after every time duration (the system responded 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.

Note that the domain lengths over which either one or two foci were consistently found are different in the two simulation methods due to stochastic effects. The stochastic case results in longer domain lengths (3 \( \mu \)m and 6 \( \mu \)m respectively) than the deterministic case (2 \( \mu \)m and 4 \( \mu \)m) for the same parameter set (compare Fig. 1D and Supplementary Fig. 2B). Intermediate domain lengths can have one or two foci dependent on the initial conditions in the continuous case or stochastic switching between one or two positioned foci in the stochastic case. The domain lengths used are chosen to differ by a factor of two as we also discuss domain growth (doubling), which has the effect of locking in a particular wavenumber \( k \).

Pattern analysis. Linear stability predicts that the solution around the fixed point is rendered spatially unstable due to the presence of diffusion and is composed of a discrete set of fundamental modes of the form \( \cos(kx/L) \). The integer \( n \) is the mode number \( (k=n\pi/L) \) is the wavenumber) and we denote the sign of the amplitude of this mode with a superscript, for example, mode 2\(^{+} \) has a peak at mid-domain, whereas mode 2\(^{-} \) has a valley at mid-domain (peaks on the boundary). Note that only even modes with negative amplitude correspond to interior (regularly positioned, symmetric) peaks. We refer to odd modes and even modes with positive amplitude as boundary modes. The growth rate of each mode around the homogeneous fixed point is given by the dispersion relation and the fastest growing unstable mode is expected to dominate the final pattern\(^{56} \). However, higher-order perturbations and multiple unstable modes can result in this prediction not holding\(^{57} \). For the chosen parameters over a 2 \( \mu \)m domain, linear stability would predict that the mode \( n=4 \) (wave number \( k_4=6.5 \) ) will dominate in both the reduced and full models (Supplementary Fig. 1b, red line). However, we find that the mode 2\(^{-} \) pattern is selected, robustly in the stochastic case (Fig. 1D). In Supplementary Fig. 1b, the first 10 (relative) amplitudes of the mode expansion are shown for some example patterns over 2 \( \mu \)m (deterministic cases) and 3 \( \mu \)m (stochastic case) domains of the full deterministic models are largely composed of only even modes with the largest contribution coming from mode 2\(^{-} \) (see also Fig. 1D).

Evidently, the presence of higher-order perturbations and/or the range of unstable modes mean that the linear approach cannot be used to predict the long-term behaviour of the system. Indeed higher-order effects are supported by the observation that at the very beginning of the simulation, the pattern is often consistent with the predicted mode and before subsequently changing. A mathematical analysis taking these higher-order effects into account is beyond the scope of this work but we note that some progress has been made in that direction\(^{58} \). Nonlinear analyses have also been used to study far-from-equilibrium solutions of reaction–diffusion equations. For example, spike or pulsed solutions have been studied extensively in the limit of asymptotically large diffusion ratio \( D_L/D_H \) (see ref. 59 for a review). Here we are interested in a system with a 25 \( \times \) diffusion ratio. The solution peaks are broader and are composed of just a few modes, unlike the narrow spike solutions. Furthermore, while the shape of a particular spike solution is not sensitive to the initial perturbation that created it, which particular solution is selected does depend on the initial conditions, unlike the case studied here.

Reduced model. In the reduced model, we set all binding and unbinding rates to zero and started the (two-variable) simulations with the same number of bound species as in the full system. This can equivalently be seen as taking the limit \( \epsilon \to 0 \), \( \delta \to 0 \), while keeping the ratio \( \epsilon/\delta \) fixed. Furthermore, it is not difficult to see that since \( \gamma \gg \delta \), the fixed points of the two systems are almost identical. As described in the text, correct positioning was no longer obtained in either the deterministic or stochastic simulations (Supplementary Fig. 2). Biochemical complexity would have to be such that \( \delta \) and \( \gamma \) are much smaller than \( \delta \), which is unrealistic for our model. The prediction from linear stability theory that mode \( n=4 \) should be dominant (over a 2 \( \mu \)m domain) also appears not to hold (Supplementary Fig. 2b, left panel, red line). The final pattern can consist of a peak or peaks anywhere on the domain although with higher likelihood away from the boundary (Supplementary Fig. 4). Note that the dominant mode is not a good descriptor of the pattern in this case as the pattern is generally the sum of many modes, both even and odd (Supplementary Fig. 2b, left panel, blue lines). In the full and reduced model, a peak was mostly situated at one of boundaries, often jumping between them (Supplementary Fig. 4). In the full system however, the flux-balance mechanism evidently shrinks the basin of attraction for the boundary patterns so that they occur less frequently in the deterministic model, while in the stochastic model, the stochastic effects allow the system to jump out of these ‘local minima’ and maintain correct positioning (Fig. 1 and Supplementary Figs 5 and 6).

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Stability of boundary modes. To investigate this stability further, we spatially perturbed the patterns obtained in the full deterministic model (over a 2 μm domain) and let them re-stabilize. We did this by cyclically translating the profile of \( v_n \), by 0.25 μm. Non-spatial perturbations, in the sense of modifying the relative concentrations of \( u_n, v_n \) and \( u \), at each spatial coordinate without mixing between the spatial locations, had little effect. We found that after a boundary focus \((n = 1)\) was shifted inwards, it re-stabilized at mid-domain \((n = 2)\), whereas a mid-domain focus returned to mid-domain. Thus, the spatial perturbation leads to selective mode transitions consistent with the observed behaviour in stochastic simulations.

Bifurcation analysis. To investigate the stability of the mode 2° and 4° patterns and the transition between them, we performed a bifurcation analysis. We treated the length as a bifurcation parameter, increasing or decreasing it in small steps (0.01 μm), starting from either the 2° or 4° pattern. At each step the solution was rescaled to match the domain length and the system solved for 60 min. We then recorded the dominant mode as for the pattern analysis. For measuring the dependence of the one-to-two peaks bifurcation on the diffusion rates, we used a step size of 0.1 μm.

Parameter sensitivity. The model has two unconstrained parameters that are important for development of a Turing pattern, namely, \( \beta \) and \( \gamma \). We therefore explored the range over which these parameters result in pattern formation in the stochastic model (the Turing space). We found that patterns were possible at least an order of magnitude around our chosen values (the coloured region in Supplementary Fig. 1c). We also examined the nature of the patterns. We calculated the first 20 wave mode amplitudes of the pattern for different \( \beta \) and \( \gamma \). We found that numerically sufficient over the parameter range investigated. In Supplementary Fig. 1c, we have indicated the dominant (non-zero) mode and the fraction of the spectral density associated with it. The dominant mode contributes to the resultant pattern for the deterministic model in the presence of cytosolic exchange than without (middle row), indicating a pattern more similar to an individual mode. However, odd dominant modes, or even dominant modes with positive amplitude, still sometimes occur (as discussed in the text and Fig. 1 and Supplementary Fig. 2). In the stochastic model, even modes with negative amplitude dominate indicating regular positioning.

Experiments. Strain AB45 (AB1157 MukB-GFP) was grown in M9 minimal medium supplemented with 0.2% glycerol and 0.2% Casamino acids at 30 °C. Fresh medium was inoculated from overnight cultures and grown for approximately 4 h to an OD of 0.1. To stain the nucleoid Sytox Orange (Thermo Fisher Scientific) was added to an aliquot at a final concentration of 500 nM and the cells were allowed to grow for a further 20 min. Cells were placed directly (without washing) on a microscopy depression slide prepared with medium and 1% agarose. Imaging was performed on a Nikon Ti-E upright microscope with a 100 × NA 1.45 phase contrast objective and an Andor Xyla sCMOS camera. During snapshot acquisition, z-stacks of phase contrast and fluorescent images were taken with 200 nm spacing (6 slices) and with a 300 nm z-offset between the phase contrast and fluorescent (GFP and Sytox Orange) channels. These stacks were then combined into single images with a higher depth of field using the ImageJ Extended Depth of Field plugin. Image segmentation and signal profile extraction was performed using Microbetracker. Matlab was used for the subsequent analysis. Signal profiles were first normalized by area to convert them to intensities (corresponding to protein concentration). To make different cells comparable, we then normalized the intensity profile by the total intensity of the cell.

Averaged demographics. MukB-GFP foci interconvert reversibly and dynamically within a time frame of less than 5 min (ref. 32). This behaviour made it difficult to ascertain when precisely a cluster had irreversibly split using time-lapse experiments (Supplementary Fig. 9a–c), especially when we wanted to relate this to the time at which the nucleoid becomes bi-lobed, an event that is also initially dynamic and reversible. Furthermore, photobleaching restricted the frame rate of time-lapse experiments to no faster than a frame every 3 min. We therefore took a demographic approach, imaging many cells with high image quality and extracting the MukB-GFP fluorescent profile along the long cell axis. Profiles from cells having the same length, to the nearest two pixels (130 nm), were then averaged and arranged into an averaged demograph (Fig. 3b).

To determine whether nucleoid segregation begins before or after MukB-GFP clusters splits, we use the DNA stain Sytox Orange to stain the nucleoid. Time-lapse experiments indicated that these two events are reasonably synchronous, but as discussed above, their dynamic nature and the restricted frame rate made it difficult to draw any further conclusion. We therefore used the average demograph approach described above to obtain the average behaviour (Fig. 3d).

Data availability. The data that support the plots within this paper and other findings of this study are available from the corresponding author on request.

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