Molecular Cell

Organization of the *Escherichia coli* Chromosome by a MukBEF Axial Core

Graphical Abstract

**Highlights**
- MukBEF forms a chromosome axial core dependent on ATP hydrolysis
- MukBEF compacts the chromosome lengthwise while avoiding links between replichores
- MatP determines the shape of the axial core by displacing MukBEF from ter
- The displacement by MatP directs MukBEF colocalization with the replication origin

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**In Brief**
Mäkelä and Sherratt demonstrated that the MukBEF SMC complex organizes the *Escherichia coli* chromosome by forming an axial core, depending on ATP hydrolysis, from which loops of size 20–50 kb emanate. MukBEF enrichment with the replication origin is directed by MukBEF displacement from the replication termination region, directed by MatP.
Organization of the *Escherichia coli* Chromosome by a MukBEF Axial Core

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https://doi.org/10.1016/j.molcel.2020.02.003

**SUMMARY**

Structural maintenance of chromosomes (SMC) complexes organize chromosomes ubiquitously, thereby contributing to their faithful segregation. We demonstrate that under conditions of increased chromosome occupancy of the *Escherichia coli* SMC complex, MukBEF, the chromosome is organized as a series of loops around a thin (<130 nm) MukBEF axial core, whose length is ~1,100 times shorter than the chromosomal DNA. The linear order of chromosomal loci is maintained in the axial cores, whose formation requires MukBEF ATP hydrolysis. Axial core structure in non-replicating chromosomes is predominantly linear (1 μm) but becomes circular (1.5 μm) in the absence of MatP because of its failure to displace MukBEF from the 800 kbp replication termination region (ter). Displacement of MukBEF from ter by MatP in wild-type cells directs MukBEF colocalization with the replication origin. We conclude that MukBEF individualizes and compacts the chromosome lengthwise, demonstrating a chromosome organization mechanism similar to condensin in mitotic chromosome formation.

**INTRODUCTION**

In all domains of life, structural maintenance of chromosomes (SMC) complexes act on chromosomes, thereby contributing to their faithful propagation and inheritance over generations. SMC roles include individualization, compaction, segregation, and cohesion of sister chromosomes (Hassler et al., 2018; Uhlmann, 2016). A substantial body of work indicates that SMC complexes from diverse organisms topologically entrap DNA double helices into sub-compartments of the complex (Chapard et al., 2019; Hassler et al., 2018; Uhlmann, 2016). Transitions between SMC complex conformational states, powered by ATP hydrolysis, potentially allow the formation of DNA loops by capture of two segments of DNA or, alternatively, by progressive enlargement of a DNA loop from an initial stem, referred as loop extrusion (Alipour and Marko, 2012; Diebold-Durand et al., 2017; Nasmyth, 2001). Although DNA loop formation appears to underlie the action of these molecular machines, exactly how they contribute to chromosome organization remains uncertain (Hassler et al., 2018; Uhlmann, 2016).

MukBEF, the *Escherichia coli* (E. coli) SMC complex homolog, exhibits the distinctive SMC complex architecture, where MukB forms dimers, each of the monomers consisting of an ABC-type ATPase head domain and a dimerization hinge separated by a long (~50 nm) antiparallel coiled-coil region (Nolivos and Sherratt, 2014). In contrast to eukaryotic SMCs, which form heterodimers, MukB and other bacterial SMCs are homodimers. The MukB globular ATPase heads are joined by C- and N-terminal interactions of MukF kleisin with the base of the MukB head (“cap”) and the MukB “neck,” a region of coiled-coil adjacent to the head, respectively (Figure 1A) (Zawadzka et al., 2018). Engagement of the heads and MukF are required for MukB ATPase activity (Zawadzka et al., 2018) and MukBEF function *in vivo* (Badrinarayanan et al., 2012a). A distinguishing feature of MukF kleisins is that they form dimers through an N-terminal winged-helix domain, leading to the formation of dimer of dimer MukBEF complexes *in vitro* (Badrinarayanan et al., 2012a; Fennell-Fezzie et al., 2005; Nolivos and Sherratt, 2014; Rajasekar et al., 2019; Zawadzka et al., 2019). Impairment of MukF dimerization leads to a failure of MukBEF complexes to stably associate with the chromosome, defects in chromosome segregation, and anucleate cell production, similar to the phenotype of cells lacking MukBEF subunits (Danilova et al., 2007; Niki et al., 1991; Rajasekar et al., 2019). Finally, MukE is an essential accessory (KITE) protein that binds MukF and modulates MukB ATPase activity (Palecek and Gruber, 2015; Zawadzka et al., 2018).

MukBEF homologs, containing a dimeric kleisin, are confined to γ-proteobacteria and have co-evolved with a set of genes, including MatP, which binds to ~23 matS sites in the ~800 kb chromosome replication terminus region (ter) (Figure 1B) (Brézellec et al., 2006; Mercier et al., 2008). Interaction of MukBEF with MatP-matS leads to displacement of MukBEF complexes from ter (Nolivos et al., 2016). Furthermore, MukB interacts with the chromosome decatenase, topoisomerase IV (TopoIV), providing a functional link between chromosome organization and unlinking (Hayama and Marians, 2010; Li et al., 2010; Nolivos et al., 2016).

Chromosome-bound MukBEF complexes form clusters, observed as “foci” by fluorescence microscopy (Figure 1C) (Badrinarayanan et al., 2012a; Danilova et al., 2007; Nolivos et al., 2016). These clusters position replication origin (oriC) regions to either mid-nucleoid (newborn cells) or nucleoid quarter
positions (cells that have replicated and segregated their oriC regions), with the MukBEF clusters being positioned on the nucleoid by a Turing patterning mechanism (Badrinarayanan et al., 2012a, 2012b; Hofmann et al., 2019; Murray and Sourjik, 2017).

We demonstrate that after modest overexpression from the endogenous chromosomal locus, MukBEF complexes form a thin axial core to the chromosome. The formation of MukBEF axial cores is directed by ATP-hydrolysis-dependent reactions, since a MukB mutant that binds ATP but is impaired in hydrolysis formed ter-associated “foci” rather than axial cores, while overexpression of a MukB mutant that cannot bind ATP led to dispersed fluorescence throughout the cell because it fails to associate stably with chromosomes. Analysis of genetic loci with respect to the axial core indicates that the overall linear order is retained, and the chromosome is organized uniformly about the axial core, leading to the conclusion that DNA loops must emanate from the axial core. The shape of the axial core is determined by MatP as it displaces MukBEF from ter, transforming a prospective circular axial core into a linear one. Abrogation of MukBEF displacement by matP deletion led to formation of the circular axial core. The axial core is ~1,100-fold shorter than the length of the chromosomal DNA with no detectable differences to wild-type (WT) in overall chromosome morphology. Under these conditions, cells grew normally and produced no anucleate cells, indicating no impairment of chromosome segregation. To reveal a possible mechanism that would give rise to the observed structures, we used stochastic modeling and simulated the action of MukBEF as a loop-extruding machine. Our simulations generated linear or circular structures without MukBEF displacement from ter on the chromosome that recapitulated the ones from microscopy. Finally, we showed that the MukBEF displacement from ter can generate a colocalization gradient of chromosomal MukBEF complexes, whose distance from oriC is minimized and maximized from ter, as observed by microscopy in WT cells. We propose that MukBEF displacement from ter acts as an alternative mechanism to promote SMC-oriC association.

RESULTS
MukBEF Forms an Axial Core to the Chromosome
To address how MukBEF organizes the E. coli chromosome, and why MukBEF clusters colocalize with and position the oriC region (Figures 1A–1C) (Badrinarayanan et al., 2012a, 2012b; Danilova et al., 2007; Hofmann et al., 2019; Nolivos et al., 2016), we modestly overexpressed MukBEF from the endogenous mukBEF operon by replacing the native promoter with an inducible promoter, ParA. We used a strain with a functional mYpet fusion to the endogenous mukB gene and FROS region (Figures 1A–1C) (Badrinarayanan et al., 2012a, 2012b; Hofmann et al., 2019; Nolivos et al., 2016), we modestly overexpressed MukBEF from the endogenous mukBEF operon by replacing the native promoter with an inducible promoter, ParA. We used a strain with a functional mYpet fusion to the endogenous mukB gene and FROS...
Figure 2. Single MukBEF Molecules on the Chromosome

(A) Log-scale distribution of apparent diffusion coefficients (D*) from single-molecule tracking of MukBJF549 with 15 ms exposure time in WT (38,502 tracks), IO (355,560 tracks) and ∆mukE (166,991 tracks) cells. Data from 3 repeats.

(B) Percentage of molecules classified as bound (D* < 0.0875 µm²/s) in WT and IO cells. The threshold was defined by the lower 5% quantile of D* in ∆mukE. Data from (A). Error bars denote SD.

(C) Representative map of MukBJF549 tracks in WT (top) and IO (bottom) cells. Error bars denote SD.

(D) Binding or activation of MukBEF complexes on the chromosome. Example frames with 1 s exposure time of a molecule producing a clear spot when bound until it dissociates or bleaches. Survival probability distributions (1-CDF) of measured residency times in WT (10,084 tracks) and IO (14,734 tracks). The data were fitted by a double-exponential function (for comparison, an exponential fit to WT). Inset shows log-log plot of the same data. The blinking- and bleaching-corrected residency time for WT is 66.9 ± 15.3 s and for IO is 63.7 ± 13.5 s (±SEM from 4 experiments).

(Fluorescent Repressor-Operator-System) markers located near oric (ori1) and close to the center of ter (ter3) (Figure 1B) (Nolivos et al., 2016). Cells grown under constant presence of 0.2% arabino resulted in 6.3 ± 0.4-fold overexpression (±SEM) (Figure S1). Under the conditions of increased MukBEF occupancy (IO) on the chromosome, fluorescent MukBEF formed a filamentous axial core that was predominantly linear (Figures 1D and S2). The ter3 marker was depleted of MukBEF fluorescence, demonstrating that the linearity of the axial core is a direct consequence of the MatP-matS-dependent displacement of MukBEF from ter. Confirming this interpretation, ∆matP cells had predominantly circular axial cores (Figure 1E). Unlike SMCs in many bacterial species (for example, Le et al., 2013; Wang et al., 2017), MukBEF does not link chromosome arms together; instead, the ends of linear axial cores in cells with MatP present (MatP⁺) localized near L3 and R3 markers that flank the ter region (Figures 1B and 1F). As or1 is localized halfway through the axial core (Figure 1D) and ter3 is in the MukBEF-depleted region, this indicates that the MukBEF axial cores retain the linear order of the chromosome.

The MukBEF axial cores form a proteinaceous structure on the chromosome and require DNA to act as a scaffold, since a mutant deficient in DNA binding as a consequence of a failure to bind ATP, MukB1040AEF (Badrinarayan et al., 2012a), did not form a structure after overexpression (Figure S1). The formation of axial cores is also dependent on ATP hydrolysis, since a MukB514070AEF mutant that binds ATP and loads on the chromosome, but is impaired in hydrolysis (Nolivos et al., 2016) formed chromosome-associated foci near ter3 under increased occupancy, rather than axial cores (Figure S1). MukBEF IO is not detrimental to the cells, as we observed the same generation time in IO cells as in WT cells (Figure S1) and IO cells did not produce anucleate cells, which arise as a consequence of a failure in chromosome segregation, a hallmark of MukBEF defects (Figure S1). DAPI-stained nucleoids of IO cells showed no detectable morphological differences to those of WT cells (Figures 1G, 1H, and S3), consistent with overall chromosome compaction being unaffected by IO. MukBEF axial cores were also observed in transcription-inhibited cells (Nonejue et al., 2013) and in cells of increased volume after treatment with A22 (Figure S4) (Wu et al., 2019), demonstrating that formation of the axial core occurs with less molecular crowding or a different cellular shape and volume.

To estimate the number of bound MukBEF complexes forming the axial cores, we first measured the fraction of chromosome-bound MukBEF complexes using single-molecule tracking with a functional HaloTag fusion to the endogenous mukB gene (Figures 2A–2C) (Banaz et al., 2019). IO cells had 25.4 ± 3.7% (±SD) of molecules immobile (chromosome-associated), as compared to 48 ± 2.6% (±SD) in WT cells. Consequently, the occupancy of bound MukBEF complexes on chromosomes, given the 6.3-fold overexpression, was increased 3.3-fold as compared to WT cells. Together with previously measured MukBEF numbers in cells (Badrinarayan et al., 2012a), we estimate ~53 and ~175 dimer of dimers MukBEF complexes on the chromosome in WT and IO cells, respectively. Additionally, using single-molecule tracking of MukBJF549 with a 1 s exposure time, we determined almost identical residency times for the chromosome-associated MukBEF complexes in WT (64 ± 14 s) and IO cells (67 ± 15 s) (±SEM) (Figure 2D), similar to the estimate for WT cells using FRAP (Fluorescence Recovery After Photobleaching) (Badrinarayan et al., 2012a).

Association of MukBEF Axial Cores with Chromosomal Loci

To quantitatively analyze MukBEF axial cores in relation to genetic markers, we enriched for cells with completely replicated
chromosomes by incubation with serine hydroxamate, thereby avoiding bias from partially replicated chromosomes. During the treatment, cells do not initiate new rounds of replication, but most complete any ongoing rounds (Ferullo et al., 2009). In single chromosome WT and IO MatP+ cells, the brightest MukBEF locus showed stronger colocalization with ori1 than ter3 as shown by the distances (Figures 3A and 3B), whereas there was no difference in colocalization of MukBEF with ori1 and ter3 in ΔmatP cells (Figures 3C and 3D). Therefore, even though the MukBEF foci in WT cells are replaced by the axial cores after IO, association of ori1 with WT cells are replaced by the axial cores after IO, association of ori1

Figure 3. Quantitative Analyses of MukBEF Localization

(A–D) Distances between the brightest MukB pixel and ori1/ter3 markers (±SEM) in (A) increased MukBEF occupancy (IO) (5,463 cells), (B) WT (4,240 cells), (C) IO-ΔmatP (4,483 cells), and (D) ΔmatP (4,702 cells) cells. Prior to imaging, cells were treated with serine hydroxamate; only cells with a single chromosome were included in the analysis. Data from 3 repeats.

(E and F) Normalized MukB pixel intensity as a function of distance to ori1/ter3 in asynchronous populations of (E) MatP+ and (F) ΔmatP cells with WT and increased MukBEF occupancy (WT, 11,567; IO, 11,030; ΔmatP, 13,089; IO-ΔmatP, 8,392 cells). * and ** denote two-sample t test between background corrected MukB intensity at ori1 and ter3 for WT (p value 0.0042), ΔmatP (p value 0.5164), IO (p value 0.0039), and ΔmatP (p value 0.0996) cells. Error bars denote SEM from 3 repeats.
that the 4.64 Mbp circular chromosome of *E. coli* has a contour length of 1.58 mm, this corresponds to a ~1,100-fold lengthwise compaction along the chromosomal axis. Additionally, we measured the length of the linear axial core along the centerline in IO cells to be 1.03 ± 0.06 μm (±SEM) (Figure 4E), shorter than in IO-ΔmatP cells (two-sample t test, p value 0.0028), extending from genetic markers L3 to R3 (3.22 Mbp; 69.5% of the genome). Since L3 and R3 colocalize with the ends of the axial core, the displacement of MukBEF extends beyond the outer matS sites and ter. The lengthwise compaction in the linear axial cores (~1,100-fold) was found similar to that of the circular cores in IO-ΔmatP cells. Finally, we determined the thickness of the axial cores. The thickness (FWHM) was approximately ~130 nm (Figure 4F), close to the limit of SIM resolution (~120 nm), thereby suggesting that the actual thickness is likely less and of the same order as the dimensions of functional MukBEF complexes (Figure 1A). In combination with the estimated volume of MukBEF dimer of dimers on the chromosome with the increased occupancy (~175), we infer that there is a complex for every ~6 nm of axial core length, consistent with it being a near continuous array of MukBEF complexes. Moreover, the average length of DNA associated with each MukBEF dimer of dimer is ~22 kbp. We summarize the dimensions of MukBEF axial cores in Figure 4G.

In MatP+ cells, MukBEF complexes are displaced from the region that extends from L3 and R3 markers, including the 800 kbp ter region. To estimate the relative compaction of the MukBEF-displaced region, we measured the minimal distance between L3 and R3 markers (1.42 Mbp; Figure 1B) in asynchronous IO MatP+ cells. We observed a bimodal distribution of distances (Figure S3), indicating that this region can be either more loosely (~400-fold), or densely (~1,000-fold) compacted. This is consistent with the observation that different genetic markers in ter can localize to distant regions of the same cell (Wang et al., 2005), with Hi-C data (Lioy et al., 2018), and with analysis of cells with increased volume (Wu et al., 2019). The L3-R3 marker distance in WT and IO MatP+ cells was indistinguishable, but in ΔmatP cells, where MukBEF complexes are not displaced from ter, L3 and R3 markers are less separated than in WT (Figure S3), showing that MukBEF action reduces flexibility in ter. As such, MatP has an important role in directing the chromosome arms to different cell halves in *E. coli*.

**Figure 4. 3D-SIM Analysis of MukBEF Axial Cores**

(A–C) Representative SIM images of increased MukBEF occupancy (IO) in (A) MatP+ with ori1 and ter3, (B) MatP+ with L3 and R3, and (C) ΔmatP with ori1 and ter3 cells. 3D-SIM images were projected onto 2D for visualization. Scale bars: 1 μm. (D) Distribution of axial core contour lengths (n = 986, ±SEM) measured from IO-ΔmatP cells. (E) Distribution of linear axial core lengths (n = 971, ±SEM) measured from IO cells. (F) Distribution of MukBEF axial core thicknesses (n = 6,657). The peak is at 132 nm. Red line denotes the resolution of SIM. For all panels, prior to imaging, cells were treated with serine hydroxamate to prevent replication initiation. Data from 3 repeats. (G) Schematic of MukBEF axial core dimensions.

**Stochastic Models of MukBEF Loop Formation on the Circular Chromosome**

How does MukBEF form the chromosome axial core? Although DNA loop formation appears to underlie the action of SMC complexes and homologs, exactly how the loops are formed remains controversial (Hassler et al., 2018; Uhlmann, 2016). Random capture of two DNA segments by MukBEF cannot generate the observed axial cores (Figure 5A). As a model, progressive enlargement of DNA loops, i.e., loop extrusion, promotes linear chromosome organization, individualization of the chromosome arms, and formation of a brush-like chromosome structure (Goloborodko et al., 2016). To understand how loop extrusion could lead to the formation of the MukBEF axial cores, we undertook stochastic simulations of the process using experimentally derived MukBEF parameters. We modeled MukBEF complexes as dimers of dimers that load randomly onto the chromosome and are capable of extruding loops bidirectionally at a rate of 600 bp/dimer/s (for comparison, rates of 100 bp/s and 1,500 bp/s were also used) (Figures 5B and S6) (Ganji et al., 2018). We assume that dimers of MukBEF complexes cannot overtake each other, and when loop extrusion

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Increased ter (48%) percentage of chromosome-bound MukBEF complexes. The loading rate was adjusted to reproduce the measured MatP- time measured here (65 s), while any complexes that encounter end the loop extrusion, MukBEF complexes spontaneously stall while the outer dimers continue to extrude loops. To brings two translocating complexes together, the inner dimers stall while the outer dimers continue to extrude loops. To end the loop extrusion, MukBEF complexes spontaneously dissociate from chromosomes after an exponential dwell time measured here (65 s), while any complexes that encounter MatP- MatS in ter are immediately released from DNA. The loading rate was adjusted to reproduce the measured (48%) percentage of chromosome-bound MukBEF complexes in WT.

We show representative simulations with (MatP+) and without (∆matP) MukBEF displacement from ter and with WT or IO numbers of bound molecules on the chromosome (Figure 5C). The chromosomes are visualized as a circular chromosome map with MukBEF-generated loops and as a force-directed layout of the chromosome that mimics “folding” of the chromosome given MukBEF-formed loops. WT MukBEF numbers results into an average loop size of 52 kbp, and the loops contain half of the chromosome (WT 48%). As MukBEF occupancy on the chromosome increases, more of the chromosome is included into MukBEF-generated loops (IO 72%) (Figure 5D), while the loop size in individual MukBEF complexes decreases (IO 30 kbp) due to more frequent collisions

WT occupancy or >1 Mbp with IO (Figures 5F and S6). By comparison, the simulations found unidirectional loop extrusion inefficient, especially in formation of larger clusters, due to gaps left behind (Figures 5D, 5F, and S6), as inferred elsewhere (Banigan and Mirny, 2019). The requirement for MukBEF to act as dimers of dimers provides a plausible mechanism for bidirectional loop extrusion (Badrinarayanan et al., 2012a; Rajasekar et al., 2019). Experimentally, the MukBEF axial core is significantly more lengthwise compacted (~1,100-fold) than the simulations of MukBEF loop extrusion predict (~10-fold). This is a consequence of the measured relatively short residency times of MukBEF complexes (~65 s); greater lengthwise compaction in the simulations would require much longer residency times. As a higher loop extrusion rate of 1,500 bp/s does not significantly affect the lengthwise compaction (Figure S6). Therefore, we propose that while MukBEF is responsible for forming the chromosome axial core that can act as a scaffold for DNA loop formation, other proteins capable of forming DNA loops or indirectly influencing chromosome compaction contribute considerably to the overall lengthwise compaction (Dillon and Dorman, 2010; Lioy et al., 2018).

Figure 5. Modeling Loop Extrusion by MukBEF
(A) Example of a simulated randomly linked E. coli chromosome.
(B) Description of the model: (a) A MukBEF dimer of dimers associates at a random site on the chromosome. (b) The loop is enlarged by the two dimers moving in opposite directions along the chromosome. (c) Collisions between MukBEFs prevent loop extrusion only on the internal collided dimers. (d) MukBEF spontaneously dissociates, releasing the loop. The MatP-bound ter region immediately displaces MukBEF upon contact.
(C) Representative E. coli chromosomes with and without MukBEF displacement from ter and WT or increased MukBEF occupancy. (top) Beginning and end of loops with MukBEF (green dots) along the chromosome. (bottom) Force-directed layouts of the chromosomes.
(D and E) (D) Fraction of the chromosome within a loop and (E) loop size per individual MukBEF as a function of the number of chromosome-bound MukBEF dimers of dimers. In unidirectional loop extrusion, dimers are not connected and each dimer acts independently, binding and extruding a loop in a randomly chosen direction. Estimated numbers for MukBEF for WT and IO cells are shown. Line thickness denotes 95% bootstrap confidence interval for the mean across 1,000 simulation replicas.
(F) Distribution of DNA in the largest MukBEF cluster (no unlooped DNA between them) for WT and IO occupancy and unidirectional loop extrusion (from the same data as in E).

(Figure 5E). The colliding MukBEF complexes form continuous clusters that can contain up to 750 kbp of DNA with
MukBEF Displacement from ter Promotes Its Colocalization with oriC

It has been proposed that MukBEF clusters are positioned autonomously at fixed positions on the nucleoid by a Turing patterning mechanism, and non-trivial interactions between MukBEF and oriC regions subsequently position oriC (Badrinarayanan et al., 2012b; Hofmann et al., 2019; Murray and Sourjik, 2017). The requirement for the accurate positioning of oriC, and consequently other chromosomal loci, is colocalization with MukBEF clusters. However, the mechanism for the observed colocalization remains unknown, given that no specific MukBEF binding sites in oriC or in the vicinity have been found. With the MukBEF loop extrusion model presented here, we considered whether it explains the colocalization in WT cells (in IO cells, ori1 is always localized halfway through the axial core) (Figure 3B) (Danilova et al., 2007). We used the largest MukBEF cluster as a proxy for the brightest MukBEF foci and computed the shortest distance along the model chromosome between a locus and the largest MukBEF cluster with WT occupancy (Figure 6A). DNA loops formed by MukBEF shorten the distances by bridging distant chromosome segments. The results show a minimum distance between the largest MukBEF cluster and the oriC region, from which the distance gradually increases until reaching the maximum distance in the middle of the ter region (Figure 6B). This is a direct consequence of MatP-matS depleting MukBEF from ter (Figure S6), which is diametrically opposite to oriC. Concomitantly, oriC is in the center of the MukBEF-occupied region, and the minimum distance always forms opposite of the depletion region. The absence of MukBEF displacement from ter (∆matP) abrogates this effect, resulting in uniform distances with all chromosome loci (Figure 6B). The profiles resemble the radial intensity patterns in IO-MatP+ or ∆matP cells showing the overall MukBEF occupancy along the chromosome (Figure S5). Different loop extrusion rates (100 bp/s and 1,500 bp/s/dimer) recapitulated the overall patterns, albeit with different overall distances (Figure S6).

In agreement with the simulations, WT cell distances from the brightest MukBEF foci were shortest at ori1 and longer at ter3 (Figure 6C). L3 showed an intermediate distance as predicted by the model. Unexpectedly, distances to R3 were greater than to ter3. Although we do not know the reason for this, it is likely to be a consequence of the behavior of FROS marker itself, as a similar effect was observed in a previous study of genetic marker positioning (Wang et al., 2006). Nevertheless, as predicted by the simulations, in ∆matP cells the distances decreased with all other markers except ori1, from which distances actually increased, as predicted by the model. MukBEF clusters colocalized almost equally with ori1, L3, and ter3, concluding that, indeed, MatP-matS-directed MukBEF displacement from ter can generate the observed oriC colocalization pattern (Danilova et al., 2007). We note that the proposed mechanism for the MukBEF-oriC colocalization pattern on the chromosome is not dependent on loop extrusion per se, and other linear chromosome organization mechanisms could reproduce the colocalization pattern. The crucial component for forming the colocalization gradient is MukBEF displacement from ter, diametrically opposite to oriC on the circular chromosome.

DISCUSSION

By using rigorous analysis of quantitative and super-resolution imaging data, we have demonstrated that under modest increased chromosome occupancy, MukBEF forms a proteinaceous axial core to the E. coli chromosome, dependent on MukBEF ATP hydrolysis. The axial core is ~1,100 times shorter than the length of the chromosomal DNA, with thickness of the same order as individual MukBEF complex dimensions. Because the overall linear order of the chromosome was retained with respect to the axial core, the only possible organization has loops of various sizes emanating from this axial core. These findings suggest strongly that MukBEF has a direct architectural role in E. coli chromosome organization. Previously, it has been inferred that MukBEF promotes DNA-DNA contacts in the range of several hundreds of kb (Lioy et al., 2018) and that clusters of its complexes, found at specific locations on the nucleoid, position origins of replication (Badrinarayanan et al., 2012b; Hofmann et al., 2019). Entropic and/or cumulative
effects of MukBEF action in chromosome segregation and organization have also been discussed (Jun and Wright, 2010). Nevertheless, there has been no premise that, in addition to any singular MukBEF activity, the emergent behavior of a higher-order structure organizes the chromosome, as revealed here. To form an axial core, sufficient MukBEF density on the chromosome is required, and we expect the MukBEF axial cores in WT cells to be more granular, less continuous entities, but with a comparable level of chromosome compaction in the MukBEF clusters.

MukBEF axial cores are at least superficially similar to “vermicelli” chromosomes observed in mammalian cells when cohesin occupancy was increased by impairing WAPL, which stimulates ATP-hydrolysis-dependent cohesin removal from chromosomes (Tedeschi et al., 2013). Similarly, the axial scaffolds formed by condensin II during the early stages of mitotic chromosome formation resemble those here (Gibcus et al., 2018). We propose that SMC complexes universally form a proteinaceous structure on the chromosome from which DNA loops emanate. Nevertheless, the molecular mechanisms that direct the formation of the structures remain elusive. Stochastic entrapment of DNA double helices, interactions between topologically (or non-topologically) loaded complexes, and convergence of the complexes during loop extrusion can, in theory, all generate the observed SMC scaffolds, and these processes do not have to be mutually exclusive (Cheng et al., 2015; Hassler et al., 2018; Uhmann, 2016). A simple model of random stepping on a thermally fluctuating DNA, which is consistent with the observations of Ganji et al., (2018), has been presented in Lawrimore et al., (2017). In directing and limiting SMC action to the same DNA molecule, loop extrusion has clear attractions, as it promotes linear chromosome compaction and individualization of the chromosome arms (Gojoborodko et al., 2016).

Individualization and separation of chromosome arms by MukBEF contrasts to the situation in Bacillus subtilis and Caulobacter crescentus, where the action of their SMC complexes zips up the two chromosome arms rather than separating them, resulting in the two chromosome arms being colinear along the cell long axis (Le et al., 2013; Wang et al., 2017). We surmise that the putative SMC-directed loops could still form within a chromosome arm, with higher-order interactions bringing the two arms together. Despite the different outcomes in overall chromosome organization between E. coli and B. subtilis, the action of their SMC complexes at the molecular level in generating DNA loops is likely to be similar and dependent on ATP hydrolysis (Wang et al., 2018). A possible analogy is the differential roles of cohesin in chromosome organization prior to S phase and its role in linking sisters after replication (Uhmann, 2016).

The circular chromosome has been imaged in shape-modified E. coli, where the larger cellular volumes constrain chromosome conformation less (Wu et al., 2019). In enlarged WT cells, DNA density varied along the chromosome with the lowest density in ter, while in enlarged ∆matP cells, the density was found to be more uniform, consistent with our observations of MukBEF axial cores in normal-shaped cells. These observations support our proposal of MukBEF being a main organizer of the chromosome and the role of MatP as a regulator of MukBEF action. The more granular, less continuous WT MukBEF axial core that we propose could be reflected in the observed high-density DNA regions, whose location and number were found to be highly dynamic in shape-modified cells. It would therefore be insightful to analyze the influence of MukBEF impairment on chromosome density in increased-volume cells.

MukBEF action on the chromosome is shaped by the chromosome-associated MatP, which plays a key role in generating the distinctive E. coli chromosome organization. First, the displacement of MukBEF from ter by MatP promotes association with the oriC region. This contrasts with the strategy in most characterized bacteria that preferentially load their SMC complexes at oriC-proximal ParB-parS sites (Gruber and Errington, 2009; Wang et al., 2017). Our results provide no support for the presence of a MukBEF loading site in the oriC region (or indeed elsewhere), since no enrichment was observed in axial core intensity toward ori1 (or ter3) upon matP deletion. We propose that MukBEF complexes can load equally well on all regions of the chromosome. Second, by deterring formation of long-range DNA-DNA interactions through MukBEF displacement (Lioy et al., 2018), MatP creates a flexible ter domain that can be present in distant regions of the same cell (Wang et al., 2005). In WT E. coli, the chromosome arms are directed to different cell halves (Wang et al., 2006), but in ∆matP cells, lacking a flexible ter region, the replicochrome separation into opposite cell halves is less pronounced, as illustrated by the smaller distances between L3 and R3 markers. Separation of the replicochromes may minimize the opportunities for chromosome entanglement and knotting, for example, by the inappropriate action of TopoIV. Third, the precocious early separation of newly replicated ter in ∆matP cells has been proposed to result from the increased abundance of MukBEF and TopoIV in ter in these cells (Nolivos et al., 2016). Still, the precise mechanism of MukBEF displacement remains to be uncovered. MatP interacts directly with the MukB dimerization hinge (Nolivos et al., 2016), but as matS sites are spaced every ~40 kbp and MatP does not spread from matS sites (Mericier et al., 2008), each MatP-matS complex must exert its effect distant from its site of binding on the chromosome, since the whole of ter is depleted for MukBEF complexes. We favor a process in which actively translocating MukBEF complexes dissociate from DNA when they encounter MatP-matS complexes, consistent with the observation that an ATP-hydrolysis-impaired MukB mutant remains associated with matS sites and ter (Nolivos et al., 2016). This also shows that the interaction of MukB with MatP-matS can occur in the absence of translocation but does not necessarily lead to dissociation from DNA.

Why does impairment of ATP-hydrolysis-dependent MukBEF action lead to defective segregation and the production of anucleate cells? In our opinion, lack of recruitment and catalytic activity stimulation of TopoIV by the MukB hinge interaction (Hayama and Marians, 2010; Li et al., 2010) is responsible for at least some of this phenotype. For example, delayed separation of newly replicated oriCs in Muk− cells is thought to be a consequence of slower decatenation because of the absence of TopoIV from MukBEF clusters (Wang et al., 2008). Consistent with this, mukBEF deletion lowers the...
chromosome-bound fraction of the TopoIV subunit, ParC (Zawadzki et al., 2015). Since TopoIV, and likely MukBEF, are “multigate” protein complexes, it is possible that coordination between their actions occurs in vivo, consistent with the observed stimulation of TopoIV catalytic activity in the presence of MukBEF. The observed MukBEF axial cores are likely to be enriched for TopoIV, whose decatenase activity will then be directed to the bases of the loops emanating from the core. Cooperation in the action of SMC complexes and type II topoisomerases may also occur in other systems (Coelho et al., 2003; Uhrmann, 2016).

We conclude that chromosome-associated MukBEF complexes are the template and “machine” for formation of DNA loops in chromosomes, and their characterization here adds weight to the hypothesis that lengthwise compaction by intra-chromosome loop formation is the mechanism by which all SMC complexes organize and individualize chromosomes.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.molcel.2020.02.003.

ACKNOWLEDGMENTS

The Micron imaging unit provided microscopes, and Lothar Schermelleh provided advice and expertise with SIM. We thank other members of the Sherratt group, Kataryzna Ginda-Mäkelä, Sophie Nolivos, Béla Novák, and Stephan Uphoff for insightful discussions. Sophie Nolivos undertook early experiments on the interplay between MukBEF-MatP-matS. Luke Lavas (Janelia Farm) provided the Halo ligand dye JF549. Wellcome (Investigator Award to D.J.S.; 200782/216/2) funded the research.

AUTHOR CONTRIBUTIONS

J.M. and D.J.S. devised and directed the research. J.M. undertook and analyzed experiments and did the simulations. J.M. and D.J.S. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.
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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial Strains** |        |            |
| *Escherichia coli* K12 AB1157 | Bachmann, 1996 | CGSC#1157 |
| AB1157 (Ab248) mukB(D1406A)-mYpet-kan | Badrinarayanan et al., 2012a | N/A |
| AB1157 (SN191) lacO240-hyg at L3 (2268 kb) tetO240-gene at R3 (852 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt mukB-mYpet-frt | AB1157 (SN191) lacO240-hyg at L3 (2268 kb) tetO240-gene at R3 (852 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt mukB-mYpet-frt | Lab collection | N/A |
| AB1157 (SN192) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt mukB-mYpet-frt | Nolivos et al., 2016 | N/A |
| AB1157 (SN301) lacO240-hyg at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt mukB-mYpet-frt | Nolivos et al., 2016 | N/A |
| AB1157 (SN311) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt mukB(E1407Q)-mYpet-frt | Nolivos et al., 2016 | N/A |
| AB1157 (JM41) mukB-HaloTag-kan | Banaz et al., 2019 | N/A |
| AB1157 (JM65) mukB-HaloTag-frt ∆mukE::kan | This study | N/A |
| AB1157 (JM90) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt kan-araC-P_{ara}-smtA-mukFE-mukB-mYpet-frt | This study | N/A |
| AB1157 (JM91) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt kan-araC-P_{ara}-smtA-mukFE-mukB-mYpet-frt | This study | N/A |
| AB1157 (JM97) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt frt-araC-P_{ara}-smtA-mukFE-mukB(E1407Q)-mYpet-kan | This study | N/A |
| AB1157 (JM101) lacO240-hyg at L3 (2268 kb) tetO240-gene at R3 (852 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt kan-araC-P_{ara}-smtA-mukFE-mukB-mYpet-frt | This study | N/A |
| AB1157 (JM103) frt-araC-P_{ara}-smtA-mukFE-mukB-HaloTag-kan | This study | N/A |
| AB1157 (JM124) lacO240-hyg at ori1 (3908 kb) tetO240-gene at ter3 (1644 kb) ∆leuB::P_{lac}-lac-l-mCherry-frt ∆galK::P_{lac}-tetR-mCerulean-frt araC-P_{ara}-smtA-mukFE-mukB(D1406A)-mYpet-kan | This study | N/A |

### Chemicals, Peptides, and Recombinant Proteins

| Chemical, Peptide, Recombinant Protein | Manufacturer | Part Number |
|--------------------------------------|--------------|-------------|
| DL-serine hydroxamate | Sigma-Aldrich | Cat#S4503 |

*(Continued on next page)*
LEAD CONTACT AND MATERIALS AVAILABILITY

The strains generated in this study are available without restriction. Further information and requests for resources should be directed to the Lead Contact, David J. Sherratt (david.sherratt@bioch.ox.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacterial strains and growth conditions

Bacterial strains and primers are listed in Key Resources Table and Table S1, respectively. All strains were derivatives of *E. coli* K12 AB1157 (Bachmann, 1996). *kan*, *cat*, *gen*, and *hyg* refer to insertions conferring resistance to kanamycin (Km r), chloramphenicol (Cmr), gentamycin (Gm r) and hygromycin B (Hyg r), respectively. The insertions are flanked by Flp site-specific recombination sites (*frt*) that allow removing the resistance gene using Flp recombinase from plasmid pCP20 (Datsenko and Wanner, 2000). To replace the native *smtA-mukBEF* promoter with an inducible *araC-Para*, a sequence containing the *kan* resistance gene and *araC-Para* from the pBAD24 (Guzman et al., 1995) was constructed. Subsequently, the native promoter of *smtA-mukBEF* operon in strain SN192 was replaced by *kan-araC-Para* using *l*-red recombination (Datsenko and Wanner, 2000). Finally, the generated chromosomal gene locus was transferred by phage P1 transduction to SN192 yielding strain JM90. P1 transduction was also used to introduce *kan-araC-Para-smtA-mukFE-mukB-mYpet* into SN302, and SN191 resulting in strains JM91, and JM101, respectively. JM103 was constructed by first removing the *kan* resistance gene from JM90 using Flp recombinase, introducing *mukB-HaloTag-kan* from JM41 by *l*-red recombination and transferring the generated chromosomal gene loci into AB1157 by P1 transduction. The JM56 strain was constructed by removing the *kan* resistance gene from JM41 and replacing the endogenous *mukE* gene with a kanamycin cassette using *l*-red recombination. The JM97 strain was constructed by introducing *mukB(E1407Q)* mutation from SN311 by *l*-red recombination into JM90 (after Flp recombinase), and verified by sequencing. The JM124 strain was constructed by introducing *mukB(D1406A)* mutation from Ab246 by *l*-red recombination into JM90 (after Flp recombinase), and verified by sequencing. All genetic modifications were verified by PCR, the Muk−/− phenotype was verified by temperature-resistance or lack of it in rich media, and behavior in quantitative imaging, as described (Nolivos et al., 2016).

Cells were grown in M9 minimal medium supplemented with 0.2% (v/v) glycerol, 2 μg ml−1 thiamine, and required amino acids (threonine, leucine, proline, histidine and arginine - 0.1 mg ml−1) at 30 °C. For MukB overexpression strains, cells were additionally grown with a constant presence of 0.2% (w/v) L-(+)-arabinose. For microscopy, cells were grown overnight, diluted 1000-fold and grown to an A600 of 0.05–0.2. For anucleate cell percentages and DAPI intensity profile analysis, cells were stained with 1 μg/mL DAPI. Cells were then pelleted, spotted onto an M9 glycerol 1% (w/v) agarose pad on a slide and covered by a coverslip. For PALM microscopy,
0.17 mm thickness coverslips were plasma-cleaned of any background fluorescent particles before use. MukB-HaloTag was labeled with JF549 ligand (Grimm et al., 2015) as in (Banaz et al., 2019).

For experiments in which cells were enriched for completed non-replicating chromosomes, cells were treated with DL-serine hydroxamate (SHX) (final concentration of 1 mg ml⁻¹). During the treatment, cells do not initiate new rounds of replication, but most complete any ongoing rounds (Ferullo et al., 2009). To allow sufficient time for ongoing replications to complete, cultures were grown in the presence of SHX for 3 h prior to imaging. This facilitated analysis of MukBEF axial cores and their distance relationships to genetic markers, because ongoing replication can bias results toward smaller ori1 distances, as the number of ori1 is greater than number of ter3 (MatP⁺, ori1/ter3 ratio 1.6; ΔmatP, ori1/ter3 ratio 1.5).

**METHOD DETAILS**

**Epifluorescence microscopy**
Fluorescence images were acquired on an inverted fluorescence microscope (Ti-E, Nikon) equipped with a perfect focus system, a 100× NA 1.4 oil immersion objective, a motorized stage, an sCMOS camera (Orca Flash 4, Hamamatsu), and a temperature chamber (Okolabs). Exposure times were 150 ms for mCherry and 75 ms for mCerulean using an LED excitation source (Lumencor SpectraX).

Cell outlines, overall and per pixel MukB-mYPet fluorescence intensities, and FROS marker coordinates were detected using SuperSegger (Stylilandou et al., 2016) in MATLAB (MathWorks). The fraction of immature MukB-mYPet molecules was estimated by considering a maturation half time of 11.9 min for mYPet at 32°C (Balleza et al., 2018), cell generation time of 116 min, and that the MukB-mYPet expression level is in equilibrium. Consequently fluorescent molecules represent a fraction 1/ (1+11.9 min/116 min) = 91% of the total MukB abundance. For fluorescence intensity profiles as a function of ori1/ter3 distance (Figure 3E, F, Figure S3), cell pixel intensities were normalized by subtracting the average cell intensity and dividing by the maximum intensity. The distance from each pixel to the closest ori1 and ter3 markers were measured and the average intensity as a function of distance was calculated. For measurement of the angle between ori1/ter3 markers and angular intensity profile for MukB (Figure S8), the center of the MukBEF structure was determined by separating MukB pixels belonging to the structure from the cellular background using Otsu’s thresholding (Otsu, 1979) and the center of mass of the region was estimated using regionprops (MATLAB). The angle between ori1/ter3 markers was then calculated using the center of the structure. The angular intensity profile for MukB was measured by dividing the structure into 45 sectors using the center of the structure as the center point, calculating the background subtracted MukB structure pixel intensity in each sector, normalizing the intensities by the average sector intensity, and finally aligning the radial profile according to the ori1/ter3 markers (Figure S5). For DAPI profiles (Figure S3), fluorescence intensity along the long cell axis for each cell was extracted. Only cells below 2.6 μm long were considered to avoid cells with more than one chromosomes. Both cell length and maximum fluorescence intensity were normalized, and the profiles were overlaid. DAPI area length was measured as full-width half-maximum of the DAPI profile.

**Photoactivated localization microscopy**
Live cell single-molecule-tracking photoactivated localization microscopy (PALM) was performed on a custom-built total internal reflection fluorescence (TIRF) microscope built around the Rapid Automated Modular Microscope (RAMM) System (ASI Imaging) with a motorized piezo stage, a z-motor objective mount, and autofocus system (MS-2000, PZ-2000FT, CRISP, ASI Imaging). MukB-HaloTag labeled with JF549 ligand was measured with a 100 mW 561 nm laser with 15% transmission (iChrome MLE, Topica). The laser was collimated and focused through a 100× oil immersion objective (NA 1.4, Olympus) onto the sample using an angle for highly inclined thin illumination (Tokunaga et al., 2008). Fluorescence emission was filtered by a dichroic mirror and notch filter (ZT405/488/561rpc and ZET405/488/561NF, Chroma). Fluorescence emission was measured using an EMCCD camera (iXon Ultra, 512x512 pixels, Andor) with a pixel size of 96 nm. Transmission illumination was provided by an LED source and condenser (ASI Imaging). PALM movies were acquired with a frame time of 15.48 ms (Banaz et al., 2019).

Single molecule tracking data was analyzed using a custom-written MATLAB software (MathWorks) as in (Banaz et al., 2019; Stracy et al., 2019). Cell outlines were detected from bright-field images as in the previous section. Fluorescently-labeled MukB were detected by using band-pass filtering and an intensity threshold to each frame of the movie. These initial localizations positions were used as a start point in a two-dimensional elliptical Gaussian fit for a high-precision localization. Fitting parameters were x-position, y-position, x-width, y-width, elliptical rotation angle, intensity, and background. Single molecule tracking was performed by linking positions to a track if they appeared in consecutive frames within a window of 0.48 μm as in (Stracy et al., 2019). In rare cases of multiple localizations within the tracking radius, tracks were linked such that the sum of step distances was minimized. Tracking allowed for a transient (1 frame) disappearance of the molecule within a track due to blinking or missed localization. The mobility of each molecule was determined by calculating an apparent diffusion coefficient, D*, from the stepwise mean-squared displacement (MSD) of the track using (Stracy et al., 2019):

\[
D^* = \frac{1}{4n \Delta t} \sum_{i=1}^{n} [x(i \Delta t) - x(i \Delta t + \Delta t)]^2 + [y(i \Delta t) - y(i \Delta t + \Delta t)]^2
\]
where \(x(t)\) and \(y(t)\) are the coordinates of the molecule at time \(t\), the frame time of the camera is \(\Delta t\), and \(n\) is the number of the steps in the trajectory. Tracks shorter than \(n = 4\) steps long were omitted due to the higher uncertainty in \(D^*\). Threshold between mobile and immobile tracks was selected by measuring \(D^*\) in \(\Delta mukE\) strain (JM56) that does not stably associate with the chromosome and setting threshold to the lower 0.05 quantile of the \(D^*\) distribution. Below this, threshold molecules were considered to be associated with the chromosome.

**Measuring long-lasting binding events**

PALM movies to measure long duration binding events of MukB-HaloTag labeled with JF549 dye were recorded using 1 s exposure times and low continuous 561 nm excitation (0.1% transmission) that blurs mobile molecules into the background whereas immobile molecules still appear as a diffraction-limited spot. Single molecule localization and tracking was used as described in the previous section. Additionally, bound and mobile molecules were distinguished by the width of the elliptical fits, with a short axis-width < 160 nm and long axis-width < 200 nm to determine bound molecules (Stracey et al., 2019) and missing frames were not allowed. The lengths of immobile tracks were measured and a survival probability curve (1-CDF) is shown in Figure 2D. To extract exponential-time constants, the survival probability curve of the immobile molecules was fitted to a double-exponential function corresponding to specific and non-specific DNA binding (Hansen et al., 2017; Rhodes et al., 2017) as a single-exponential function was found to not properly fit the survival probability curve. The fitting was performed using least-squares criterion with a weight \(1/y\) to compensate for small values in the tail. The duration of specific DNA binding events was defined by the slower rate of the double-exponential function. The probability of measuring a particular time of binding event is also influenced by the bleaching and blinking properties of the fluorescent dye. To assess the influence of these processes, along with errors in detection, the bleaching-time distributions were measured independently under the same conditions using cells fixed with 4% (v/v) paraformaldehyde that blocks molecule movement. As before, a bleaching-time survival probability curve was fitted by a double-exponential function to extract exponential-time constants. The MukB-HaloTag bleaching time constant, \(t_{bleach}\), was measured to be 48.8 ± 8.3 s (9739 tracks; ± SEM from 3 experiments). The bleaching corrected binding-time was calculated by \(t_{bound} = t_{measured} \times t_{bleach} / (t_{bleach} - t_{measured})\) (Rhodes et al., 2017). Blinking of fluorescent dye before or during binding events does not influence the measurement, because the observed binding times follow an exponential distribution and are therefore memoryless. All data analysis was performed in MATLAB (MathWorks).

**3D-structured illumination microscopy**

Super-resolution 3D-structured illumination microscopy (SIM) images were acquired on a DeltaVision OMX V3 Blaze instrument (GE Healthcare), equipped with a 60×/1.42 oil UPlanSApo objective (Olympus), 405 nm, 488 nm and 593 nm diode lasers and three sCMOS cameras (PCO). Multi-channel three-dimensional stacks of MukB–mYpet/TetR-mCerulean were imaged sequentially. For each channel, the raw 3D-SIM stacks were composed of 225 512x512 pixel images consisting of 21 z sections (125 nm z-spacing, sample thickness of 2.5 mm). Each section consisted of 15 images - 3 angles and 5 phase shifts. Additionally, Lacl-mCherry was imaged in a conventional wide-field mode. Acquisition settings were as follows: MukB–mYpet, 20 ms exposure with 488 nm laser (attenuated to 30% transmission); TetR-mCerulean, 50 ms exposure with 405 nm laser (30% transmission), Lacl-mCherry, 50 ms exposure with 593 nm laser (30% transmission). The 3D-SIM raw data was computationally reconstructed with SoftWoRx 6.0 (GE Healthcare) using a Wiener filter setting of 0.004 and channel specific optical transfer functions to generate a super-resolution three-dimensional image stack with a lateral (x-y) resolution of ~120nm (wavelength dependent) and an axial (z) resolution of ~300 nm. In the reconstruction process, the pixel size was halved from 80 nm to 40 nm and the pixel number doubled in order to meet the Nyquist sampling criterion. For multichannel 3D alignment, mouse C127 cells were three-color (405, 488 and 594 nm) 5-ethenyl-2'-deoxyuridine (EdU) pulse-labeled as described in (Kraus et al., 2017). The multichannel 3D-SIM EdU foci images were captured and reconstructed as described above, then channels were 3D aligned and corrected for chromatic shifts using the open-source software Chromagnon (Matsuda et al., 2018). The correction parameters obtained were then applied to align images from the experiments. ImageJ (Schneider et al., 2012) plugin 3Dscript (Schmid et al., 2019) was used to generate 3D rendering of MukB–mYpet signals (Videos S1 and S2).

For analysis of MukBEF structure dimensions (Figure 4D-F, Figure S4), 3D-SIM image stacks were processed using ImageJ plug-in SIMcheck (Ball et al., 2015) and projected along the z axis and the maximum intensity for each pixel selected. Only filaments with clear orientation in x-y axes were selected for analysis. Pixels belonging to the MukBEF structure were separated from the background using Otsu’s thresholding (Otsu, 1979). The structure’s centerline was calculated by using a morphological operation (bwmorph, MATLAB, MathWorks) that erodes pixels from edges until only center pixels of the structure are left. Following this, branches of length 1 in the centerline were removed. The length of a linear structure was measured as the minimum length of a curve that includes all pixels of the centerline. The length of a circular structure was measured as the minimum contour length of a polygon that includes all pixels of the centerline. The thickness of the structure was measured by fitting a lineograph of pixel intensities across the centerline with a Gaussian function. Pixels close to the ends or branching points of the backbone were removed from the analysis. Further, the lineograph orientation was selected around a pixel to be normal to the structure so as to minimize width. From a Gaussian fit, full-width half-maximum (FWHM) distance was calculated as follows:
same everywhere on the chromosome, except in simulations with MatP dependent displacement of MukBEF from ter bound to the chromosome with wild-type MukBEF copy numbers (110 MukBEF dimer of dimers) (Badrinarayanan et al., 2012a). The loop extrusion rate ($k_{\text{move}}$) has not been directly measured.

Stochastic simulations were performed using SGNS2 (Lloyd-Price et al., 2012), which uses the Gillespie method (Stochastic Simulation Algorithm) (Gillespie, 1977) to obtain exact realizations of the Chemical Master Equation (CME). SGNS2 supports dynamic compartments that can be created or destroyed during a simulation. The circular chromosome of E. coli was divided into 4641 discrete DNA segments, with each segment corresponding to a specific 1 kbp region of the chromosome. MukBEF is, unless otherwise stated, modeled as a dimer of dimers, which randomly binds to 2 adjacent free sites on the chromosome with a stochastic rate ($k_{\text{bind}}$) with equal probability throughout the chromosome. Binding of MukBEF creates a dynamic compartment that contains a single DNA loop where each dimer of MukBEF occupies a single DNA segment. Following the binding event, each dimer of the compartment moves unidirectionally and independently away from each other one DNA segment at a time (releasing previous DNA segment while occupying the consecutive one) with a stochastic rate ($k_{\text{move}}$) for extrusion of a loop. As the MukBEF dimers move away from initial binding segment, DNA in the loop is free to be bound by other MukBEF molecules allowing for the creation of loops inside loops. If dimers collide on the chromosome head-on, they block each other, while the outer dimers of the MukBEF continue loop extrusion unperturbed. Unbinding of MukBEF releases the DNA segments under its footprint and destroys the loop with a stochastic rate ($k_{\text{unbind}}$) that is independent of the state of the chromosome or other MukBEF. The residency time is the same everywhere on the chromosome, except in simulations with MatP dependent displacement of MukBEF from ter region, where binding or moving leads to instant dissociation of MukBEF molecule and destruction of the loop. Aforementioned reactions of the model are written for every DNA segment of the system with ter segments containing also the release reaction by MatP. Asymmetric loop extrusion is modeled by only one of the dimers moving away from the binding site; orientation decided randomly at the binding. The cytosolic state of MukBEF is assumed well-mixed and is therefore treated implicitly.

The rate constants were used as measured here. Namely, the MukBEF unbinding rate ($k_{\text{unbind}}$) is 0.0154 s$^{-1}$ (65 s residency time) and the MukBEF binding rate ($k_{\text{bind}}$) is 3.9e-06 s$^{-1}$ per DNA segment per free MukBEF complex which results in 48% of MukBEF to be bound to the chromosome with wild-type MukBEF copy numbers (110 MukBEF dimer of dimers) (Badrinarayanan et al., 2012a). The loop extrusion rate ($k_{\text{move}}$) has not been directly measured in vivo and therefore was set to 0.6 DNA segments/dimer/s (corresponding to 600 bp/dimer/s) as estimated in vitro for condensin (Ganjei et al., 2018). The expected loop size without collisions is 80 kbp (40 kbp for unidirectional loop extrusion). Simulations for loop extrusion rates of 100 bp/dimer/s and 1500 bp/dimer/s were also undertaken. The system state including the state of each loop compartment was read out after 500 s to allow the overall loop structure on the chromosome to reach maturation. Each simulation was repeated at least 1000 times to ensure proper sampling of chromosome states.

In the analysis of simulated chromosomes, MukBEF clusters were defined as MukBEF complexes that do not have unlooped DNA segments between them. Loops inside loops can contribute to the cluster size if they have reached the stem of the main loop by at least from one side. After finding the largest MukBEF cluster, the shortest distance between the largest cluster and a chromosome locus was measured along the chromosome from a DNA segment of the specific chromosome locus to the closest MukBEF of the largest cluster. Loops acts as ‘shortcuts’ decreasing the distances between chromosomal loci. The loop state of a single chromosome (Figure 5C) was shown as a polar coordinate plot that shows the starting and the ending locations of DNA loops or as a 2D force-directed layout of the circular chromosome after converting the loop state into a graph with loops as connections between otherwise circular organization of DNA segments. Random links (Figure 5A) were generated by adding 52 (wild-type occupancy) connections between random DNA segments. All data analysis was performed in MATLAB (MathWorks).

Quantification and Statistical Analysis

Statistical details of experiments can be found in the figure legends. This includes exact value of samples, number of experiments and definition of dispersion measures (SD or SEM) between experiments. Microscopy images were randomly collected to obtain sufficient number of cells for each dataset. No data was excluded besides the specific criteria defined in the figure legends. Independent experiments were used to define the reproducibility of results. Two-sample unpaired Student’s t test, performed by using ttest2 function in MATLAB (MathWorks), was used for hypothesis testing of equal means and equal but unknown variances between samples. Significance was defined as p value < 0.01.

Data and Code Availability

The following data are available at Mendeley data (https://doi.org/10.17632/d7p7hk3zv.1): The raw epifluorescence data (Figures 3, 6, S1, S3, and S5); the single-molecule localizations and tracks used for D* histograms and residency times (Figure 2); and the raw data for representative images in all figures. All materials and codes are available upon reasonable request.
Supplemental Information

Organization of the *Escherichia coli* Chromosome by a MukBEF Axial Core

Jarno Mäkelä and David J. Sherratt
Figure S1

A

Fold-change

WT = 6.3 ± 0.4
ΔmatP = 6.2 ± 0.2

WT
n = 12260
ΔmatP
n = 13854
MukBEF over-expression
n = 11863
MukBEF over-expression ΔmatP
n = 8978

B

Generation times:

Wild type 116 ± 4 min
MukBEF over-expression 115 ± 3 min
MukBEF over-expression ΔmatP 116 ± 1 min

C

Anucleate cells:

Wild type (8726 cells) 0.23 ± 0.01%
ΔmatP (9223 cells) 0.66 ± 0.27%
MukBEF over-expression (24537 cells) 0.12 ± 0.06%
MukBEF over-expression ΔmatP (19409 cells) 0.59 ± 0.16%

D

Fold-change

13.1 ± 0.3

MukB1407EF
n = 24028
MukB1407EF over-expression

E

MukB1407EF over-expression

F

Fold-change

11.9 ± 0.6

MukB1406EF
n = 11103
MukB1406EF over-expression

G

MukB1406EF over-expression

H

Probability density

MukB1407EF
0.43 ± 0.01 µm
0.82 ± 0.01 µm
ori1
ori1

I

Probability density

MukB1407EF over-expression
0.36 ± 0.01 µm
0.98 ± 0.02 µm
ori1
ori1

Distance from the brightest MukB pixel to ori1/ter3 (µm)
**Figure S1.** Characterization of increased MukBEF chromosome occupancy cells and MukBEF mutants. Related to Figure 1.

(A) MukB-mYpet fluorescence intensity from 3 repeats in WT, ΔmatP, MukBEF over-expression and MukBEF over-expression ΔmatP cells and fold-change between WT and MukBEF over-expression. *n* denotes number of cells and error bars denote SD. (B) Generation times from 3 repeats (±SEM) for WT, over-expression and over-expression ΔmatP. (C) Anucleate cell percentages using DAPI from 3 repeats (±SEM) in WT, ΔmatP, MukBEF over-expression and MukBEF over-expression ΔmatP. (D) MukBE1407Q-mYpet fluorescence intensity in MukBE1407QEF and MukBE1407QEF over-expression cells. *n* denotes number of cells and error bars denote SD from 3 repeats. (E) Representative phase contrast and fluorescence images of MukBE1407QEF over-expression cells with ori1 and ter3 markers. Scale bars, 1 μm. (F) MukBD1406A-mYpet fluorescence intensity in MukBD1406AEF and MukBD1406AEF over-expression cells. *n* denotes number of cells and error bars denote SD from 3 repeats. (G) Representative phase contrast and fluorescence images of MukBD1406AEF over-expression cells with ori1 and ter3 markers. Scale bars, 1 μm. (H)-(I) Distances between the brightest MukBE1407Q-mYpet pixel and ori1/ter3 markers in (H) MukBE1407QEF (8773 cells) and (I) MukBE1407QEF over-expression cells (2313 cells). Data from 3 repeats (±SEM).
Figure S2

A

B

C
Figure S2. MukBEF increased occupancy cells in MatP$^+$ (with MatP present) and ΔmatP. Related to Figure 1.

Representative phase contrast and fluorescence images of cells with (A) MukBEF increased occupancy ΔmatP cells with ori1 and ter3 markers, (B) MukBEF increased occupancy with ori1 and ter3 markers, and (C) MukBEF increased occupancy with L3 and R3 markers. Scale bars, 1 μm.
### Figure S3

#### A

**WT:**
- Nucleoid length: $1.20 \pm 0.04 \mu m$
- Nucleoid distance to closest cell pole: $0.40 \pm 0.01 \mu m$

**IO:**
- Nucleoid length: $1.07 \pm 0.09 \mu m$
- Nucleoid distance to closest cell pole: $0.42 \pm 0.02 \mu m$

#### B

**MukBEF over-expression**
- MukBEF over-expression
- MukBEF over-expression ΔmatP
- Wild type
- ΔmatP

#### C

**MukBEF over-expression**
- MukB-ori1
- MukB-ter3
- Glucose
- 20 min
- 40 min
- 60 min
- 100 min
- 160 min
- O/N

#### D

- 0.4 μm
- 1.25 μm

#### E

**L3-R3 distance (μm)**
- IO: $1.05 \pm 0.04 \mu m$
- WT: $1.01 \pm 0.03 \mu m$
- ΔmatP: $0.68 \pm 0.01 \mu m$

**p = 7.8 \times 10^{-5}** n.s.
Figure S3. DAPI profiles, induction of MukBEF over-expression time series and L3-R3 distances. Related to Figure 1, 2, and 4.

(A) Normalized DAPI intensity profiles on normalized long cell axis for WT (871 cells) and MukBEF increased occupancy (8250 cells). Only cells below 2.6 μm long were considered to avoid cells with more than 1 chromosomes. Nucleoid length was measured as full-width-half-maximum (FWHM) of the DAPI profile. Also, the distance to the cell pole from half maximum of the DAPI profile was measured. Shaded area denotes SEM. Data are from 3 repeats. (B) MukB-mYpet intensity in wild type and MukBEF over-expression cells during induction with 0.2 % (w/v) arabinose. Fluorescence expression levels are shown prior to induction (glucose), during induction (20 min, 40 min, 60 min, 100 min, 160 min) and cultures grown over night in the presence of arabinose. Left distributions correspond to MukBEF increased occupancy MatP⁺ strain and right distributions to the MukBEF increased occupancy ΔmatP strain. Also shown are wild type and ΔmatP strains. Black lines show the means. Data are from at least 2 independent experiments with number of data points in WT (12260, 13854), glucose (9537, 4614), 20 min (8521, 9385), 40 min (11419, 5939), 60 min (8982, 8995), 100 min (9630, 10152), 160 min (9396, 10212), and over-night induction (11863, 8979) in MatP⁺ and ΔmatP cells, respectively (no. cells). (C) Normalized MukB-mYpet pixel intensity during induction as a function of distance to ori1/ter3 in (top) MukBEF increased occupancy and (bottom) MukBEF increased occupancy ΔmatP cells. Also shown are wild type and ΔmatP strain. Data are same as in (B). (D) Distances between L3 and R3 markers in MukBEF increased occupancy cells. 2824 single chromosome cells from 2 experiments were analyzed. (E) Distances between L3 and R3 markers in IO (7467 cells), WT (2444 cells) and ΔmatP (2796 cells) cells. Prior to imaging cells were treated with serine hydroxamate and only cells with a single chromosome were analyzed. Two-sample t-test was used to compare conditions. Error bars denote SD from 3 experiments.
Figure S4

A

B

C

D

E

F
Figure S4. 3D-SIM images of IO cells and IO cells treated with A22 or rifampicin. Related to Figure 1, 4 and STAR Methods.

(A) Representative SIM images of MukBEF increased occupancy ΔmatP cells with ori1 and ter3 markers grown with A22 (4 μg/ml) for 6 h. 3D-SIM images were maximum projected onto 2D for visualization purposes. Scale bars, 1 μm. (B) Distribution of axial core contour lengths (259 chromosomes) in the same conditions as in (A). (C) Representative SIM images of MukBEF increased occupancy ΔmatP cells with ori1 and ter3 markers grown with rifampicin (0.025 μg/ml) for 2 h. Scale bars are 1 μm. Representative SIM images of MukBEF increased occupancy in (D) MatP+ cells with L3 and R3 markers, and (E) ΔmatP cells with ori1 and ter3 markers. 3D-SIM images were maximum projected onto 2D for visualization. Scale bars, 1 μm. (F) Example images of detected centerlines of circular and linear the MukBEF structures in ΔmatP and MatP+ cells, respectively. White line is the cell border. Green dots are centerline pixels of the structure.
Figure S5

A

B

Majority of angles (>0.5 CDF) are within 16.3 ± 1.8 degrees from the expected value (175.6 degrees)

C

D

E

F
Figure S5. MukBEF axial core analysis. Related to Figure 3.

(A) Illustration of a circular axial core divided into sectors with ori1 and ter3 markers. Using the center of the axial core, the angle between ori1 and ter3 is measured. The axial core is divided into sectors from which the MukB intensity is calculated (in the actual measurement 45 sectors were used). (B) The minimum angle between ori1 and ter3 in IO-ΔmatP cells (1206 cells from 3 repeats). Only cells with a single chromosome (a single ori1 and ter3) and symmetric structures are included in the analysis (relative difference between long and short axis less than 0.3). Prior to imaging, cells were treated with SHX. The red line is the expected angle (175.6 degrees) between ori1 and ter3. (C)-(F) Normalized radial MukBEF intensity of axial cores in (C) IO-ΔmatP cells aligned to ori1, (D) IO-ΔmatP cells aligned to ter3, (E) IO-MatP+ cells aligned to ori1, and (F) IO-MatP+ cells aligned to ter3. Black line shows the mean with the thickness corresponding to ±SD. Colored lines are representative axial core intensities. The intensity is measured only for the pixels contained in the MukBEF structure. Prior to imaging, cells were treated with serine hydroxamate. Only cells with a single chromosome (a single ori1 and ter3) and a symmetric structure are included in the analysis (difference between long and short axis less than 0.3). Data are from 3 repeats (IO-ΔmatP 1206 cells; IO-MatP+ 1495 cells).
Figure S6

A. Fraction of loops inside circles as a function of No. MukBEF on the chromosome.

B. DNA in the largest MukBEF cluster (kbp) as a function of No. MukBEF on the chromosome.

C. Probability density of the ends of loops.

D. Distance to largest MukBEF cluster (kbp) as a function of 100 bp/dimer/s, 600 bp/dimer/s, and 1500 bp/dimer/s.

E. Loop size (kbp) and fraction of DNA in loops for WT, ΔmatP, ΔmatP, ΔmatP, ΔmatP, and ΔmatP.

F. Diagrams of 100 bp/dimer/s and 1500 bp/dimer/s.

G. Diagrams showing orC, MukBEF, chromosome, and MatP region.
Figure S6. Modeling loop extrusion by MukBEF analysis. Related to Figure 5.

(A) Fraction of loops inside loops and (B) DNA in the largest MukBEF cluster (no unlooped DNA between them) as function of the number of bound MukBEF dimers of dimers. Experimentally observed numbers of MukBEF dimers of dimers on the chromosome for wild-type (○) and MukBEF increased occupancy (◊) are depicted. Additionally, unidirectional model of loop extrusion (black line) is shown where each dimers binds and extrudes a loop independently in a randomly chosen direction. Line thickness denotes 95% bootstrap confidence interval for the mean across at least 1000 simulation replicas. (C) MukB occupancy profile on the chromosome with wild-type MukBEF occupancy across 4000 simulation replicas. (D) Shortest distance from a chromosome locus to the largest MukBEF cluster with different loop extrusion rates (100 bp/dimer/s, 600 bp/dimer/s and 1500 bp/dimer/s) with (WT) or without (ΔmatP) MukBEF displacement from ter. Line thickness denotes 95% bootstrap confidence interval for the mean across 2000 simulation replicas. (E) Average loop size and fraction of the chromosome in loops for different loop extrusion rates. Expected loop sizes without collisions are 15 kbp, 80 kbp and 197 kbp for 100 bp/dimer/s, 600 bp/dimer/s, and 1500 bp/dimer/s, respectively. 95% bootstrap confidence interval for the mean across 1000 simulation replicas in parenthesis. (F)-(G) Representative E. coli chromosomes for (F) 100 bp/dimer/s and (G) 1500 bp/dimer/s loop extrusion rates with increased MukBEF occupancy. (left) Beginning and end of loops with MukBEF (green dots) along the chromosome. (right) Force-directed layouts of the chromosomes.
Table S1. Primers used for strain construction. Related to STAR Methods.

| Name  | Sequence                                                                 | Construct                                      |
|-------|---------------------------------------------------------------------------|------------------------------------------------|
| OL1_F | GGATTCTGCAAACCCTATGCTACTCCCGG AGTGTAGGCTGGAGCTTC                           | kan-araC-Para construction using Gibasenity assembly. PCR on pBAD24 (OL3_F/OL2_R) and pKD4 OL1_F/OL4_R. |
| OL2_R | GAAGCAGCTCCAGCTACACTCCGGGAGT AGCATAGGGTTGAGAACGAGT CTCAGCTGGTGTCTGATTC   |                                                 |
| OL3_F | TAAGGAGGATATCATATGGGTAACCGTC AAGCCGTCAGTTGCTGATTC                         |                                                 |
| OL4_R | GAATCGAGACAAATGACCGGCTGACAGTTA CCCATATGAATACATCCCTGCTTA                   |                                                 |
| OL5_F | CCACACGAGCGCCAGGCAAGCAGGCAATAA TCAACAACACATCAGCGGAAGTGTAGGTGGTCTGAGCTGCTCC | λ-red replacement of Pmuk with kan-araC-Para at the endogenous locus. PCR on kan-araC-Para from Gibson. |
| OL6_R | TGATATACGTGGGCATCCTTTGCTATAT GTTAAAGAAAAAGGAGGAGAATTCTTCTGCTAGCCAAAA      | λ-red insertion of mukB-HaloTag-kan. PCR on JM41. |
| OL7_F | AATTGTTGTGAGCGCTGTTGCAAATGCA                                           | λ-red deletion of mukE. PCR on strain with ΔmukE::kan from lab collection. |
| OL8_R | GTACAGACGCAAATACTCAGCGAAAATG                                            |                                                 |
| OL9_F | CCGATTAATGATTACGGAGGAGGCA                                               |                                                 |
| OL10_R| CCCGGCTCCGTAGTTGTTACGAAA                                               |                                                 |
| OL11_F| GTATCGTTTGGTGAGCTGGAACAG                                               | λ-red insertion of mukB(E1407Q)-mYpet-kan or mukB(D1406A)-mYpet-kan. PCR on SN311 or Ab246. |
| OL12_R| GTACACGAAATATCAGCGAAAAGT                                              |                                                 |