Nonrandom segregation of sister chromosomes by *Escherichia coli* MukBEF

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Structural maintenance of chromosomes (SMC) complexes contribute to chromosome organization in all domains of life. In *Escherichia coli*, MukBEF, the functional SMC homolog, promotes spatiotemporal chromosome organization and faithful chromosome segregation. Here, we address the relative contributions of MukBEF and the replication terminus (ter) binding protein, MatP, to chromosome organization—segregation. We show that MukBEF, but not MatP, is required for the normal localization of the origin of replication to midcell and for the establishment of translational symmetry between newly replicated sister chromosomes. Overall, chromosome orientation is normally maintained through division from one generation to the next. Analysis of loci flanking the replication termination region (ter), which demark the ends of the linearly organized portion of the nucleoid, demonstrates that MatP is required for maintenance of chromosome orientation. We show that DNA-bound $\beta_{2c}$-processivity clamps, which mark the lagging strands at DNA replication forks, localize to the cell center, independent of replisome location but dependent on MukBEF action, and consistent with translational symmetry of sister chromosomes. Finally, we directly show that the older ("immortal") template DNA strand, propagated from previous generations, is preferentially inherited by the cell forming at the old pole, dependent on MukBEF and MatP. The work further implicates MukBEF and MatP as central players in chromosome organization, segregation, and nonrandom inheritance of genetic material and suggests a general framework for understanding how chromosome conformation and dynamics shape subcellular organization.

MukBEF | MatP | SMC | chromosome organization | DNA replication

**Significance**

Circular chromosomes in rod-shaped bacteria exist inside a cell in two distinct configurations, "transverse" and "longitudinal," relative to the long cell axis, with chromosomal loci occupying specific cellular locations in both cases. Bacteria with longitudinal chromosome organization (e.g., *Caulobacter crescentus*) typically tether their origins of replication to the cell membrane and do not undergo overlapping rounds of replication. In contrast, bacteria with transverse organization (e.g., *Escherichia coli*) orient their chromosomes by an unknown mechanism and have lifestyles compatible with overlapping rounds of replication. Here, we address the relative roles of two major players in chromosome organization—segregation and propose a model of how *E. coli* maintains chromosome conformation and orientation inside cells and how this organization is propagated over generations.

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The authors declare no competing interest.

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identical to their mother cell. Most cells adopt a left-oriC-right-left-oriC-right (L-R-L-R) translational symmetry prior to division (12), which requires that either the leading or lagging strand templates are symmetrically segregated to the cell poles (11, 20). In agreement, an elegant chromosome degradation experiment showed that the leading strand templates are segregated toward the cell poles in most cells (21). In theory, cells could also additionally control the fate of the old template strand by nonrandom segregation, designating the destination for each template strand. Coincided as “immortal” strand retention, it was originally proposed as a strategy to maintain DNA purity in stem cells while the copied strands, potentially carrying mutations from replication, were segregated to nonstem cell progeny (22). Whether this strategy is actually utilized by stem cells remains controversial (23–25). Immortal strand segregation has been tested in Caulobacter crescentus (26, 27) and Bacillus subtilis (28); however, none of these studies showed any segregationist strand preference between daughter cells.

We lack a mechanistic understanding of how chromosome conformation and orientation is maintained inside a bacterial cell. It also remains unknown how progressive chromosome segregation facilitates nonrandom sister chromosome inheritance in an otherwise apparently symmetrical organism. Here, we address these questions in E. coli utilizing microfluidics culturing devices, combined with time-lapse imaging, high-throughput microscopy, and quantitative analysis. We first demonstrate that in the absence of MukBEF, anucleate cells arise predominantly from the mother cell’s new pole as a consequence of the failure to segregate newly replicated origins in a timely fashion. We show that nascent lagging strands and their templates are directed toward cell centers, a process that is required for the observed translational L-R-L-R segregational symmetry and which is perturbed in the absence of MukBEF. Furthermore, we show directly that the older template DNA strand, inherited from previous generations, is preferentially segregated to the old cell pole, dependent on both MukBEF and MatP. Lack of MatP does not perturb translational L-R-L-R symmetry; rather, it leads to flipping of chromosome orientation along the longitudinal cell axis, consistent with the observed loss of the older template strand retention at old poles. Taken together, the results provide a model of how MukBEF and MatP-driven depletion from the ter region lead to asymmetric strand and chromosome segregation. The possible functional and evolutionary consequences of this are discussed.

Results

In the Absence of MukBEF, Anucleate Cells Arise from the Newer Mother Cell Pole. To understand how anucleate E. coli cells form in the absence of MukBEF, we followed the successive cell cycles of ΔmukB cells with oriC and ter (ori1 and ter3, respectively) regions fluorescently labeled by fluorescence repressor–operator system (FROS) markers. A “mother machine” microfluidics device (29, 30) allowed us to follow thousands of cell generations and identify changes in chromosome organization that correlate with chromosome missegregation (Fig. 1L and SI Appendix, Fig. S1 and Movie S1). Under the growth conditions used (M9 medium supplemented with glucose and essential amino acids at 37 °C, 15.7 ± 0.4% (±SD) of ΔmukB cell divisions led to the formation of an anucleate daughter cell, in comparison to 0.13 ± 0.01% (±SD) of WT cell divisions.

In ΔmukB cells, ori1 loci localized preferentially toward the old cell pole (Fig. 1B) (16), with the newly replicated sister ori1 loci frequently remaining in close proximity. Note that replication initiation is not significantly delayed in ΔmukB cells as compared to WT cells, in which sister ori1 separation occurs in a timely manner (18). Meanwhile, ter3 migration from the newborn cell pole to midcell was only modestly delayed in comparison to WT cells (Fig. 1B). Around ~80% of anucleate cells were generated when duplicated ori1 loci in mother cells remained together in the old pole cell half prior to cell division (Fig. 1C). In contrast, in ~70% of mother cells, in which chromosome segregation was faithful, ori1 loci were visible as separate foci. In the ~30% of ΔmukB cells (as compared to ~10% of WT cells) that had a single ori1 focus prior to division, which divided and segregated their chromosomes successfully, that single focus must have contained two unsegregated ori1 loci. This shows that ori1 numbers are undercounted in our experiments, but it does not change the fact that cells undergoing anucleate division have significantly less separated ori1 loci.

In anucleate ΔmukB cell divisions, daughter cells that inherited two chromosomes divided normally after a modest increase in generation time (normal divisions 63 ± 5 min, anucleate sisters 72 ± 4 min [±SD]; two-sample t test P value 0.2176; SI Appendix, Fig. S1A). However, the probability of these cells forming an anucleate cell in subsequent division was 9.1 ± 2% (±SD), significantly lower than for cells born with a single chromosome. During anucleate cell formation, mother cells divided nearly symmetrically (anucleate cell 2.1 ± 0.2 μm and sister cell 2.4 ± 0.2 μm [±SD]; two-sample t test P value 0.17), with the divisome being placed close to midcell. While the average anucleate cell length at birth did not significantly differ from that of the growing sister (SI Appendix, Fig. S1B), the bias for the longer growing sister increased with mother cell division size. Note that WT cells had a similar generation time (59 ± 1 min [±SD]) to the ΔmukB cells, indicating that the cell-cycle parameters of ΔmukB and WT cells are likely to be similar, as reported previously for cells growing in minimal medium at 30 °C (18).

Finally, we showed that anucleate cells form preferentially at the newer mother cell pole (74.4 ± 1.8% [±SD]; Fig. 1D). Therefore, anucleate cell formation is associated with the nucleoid being preferentially retained at the old pole of ΔmukB mother cells, while in the case of WT cells the nucleoid is localized closer to the newer pole of a dividing cell (31). We conclude that the mislocalization of ori1 loci toward the old pole and delayed segregation of newly replicated ori1 loci are linked to the formation of anucleate cells to the mother cell’s new pole.

MukBEF and MatP Have Distinct Roles in Generation and Propagation of left-oriC-right Chromosome Organization Over Generations. Next, we explored the contributions of MukBEF and MatP in dictating left-oriC-right (L-R) chromosome organization in E. coli and in the propagation of these patterns over generations (12, 17). We used strains that allowed us to test the requirements for left and right chromosome arm organization in relation to oriC and ter in WT, ΔmukB, and ΔmukB cells (Fig. 2). The left and right chromosome arms were labeled at L3 and R3 (~128° and 122° from orIC, respectively) with FROS markers, as were ori1 and ter3 loci (Fig. 2 A and B). We used M9 medium supplemented by glycerol and required amino acids at 30 °C to avoid overlapping replication; under these conditions, replication is initiated several minutes after birth and completed before cell division (18). These growth conditions were used for all experiments described in the following, unless otherwise stated.

Newborn WT cells exhibited the distinctive left-oriC-right (L3-R3) chromosome organization (Fig. 2 C–E), in which oriC remained at the cell center and the chromosome arms (assayed by L3 and R3 localization) resided in opposite cell halves (97.8 ± 0.6% [±SD]; Fig. 2 F and G) (12, 32). During replication–segregation, the pattern was extended into a translationally symmetric left-oriC-right-left-oriC-right (L3-R3-L3-R3 or R3-L3-R3-L3) pattern in 73.1 ± 3.9% (±SD) of WT cells (Fig. 2G), compared to mirror symmetric L3-R3-L3-R3 or R3-L3-R3-L3 patterns.

In the absence of MukB, the localization of ori1, L3, and R3 chromosomal markers was less precise (Fig. 2 C–E), with a wide distribution of L3-R3 distances (Fig. 2F), fewer L3 and R3 foci localizing in opposite cell halves (56.6 ± 3.2% [±SD]; Fig. 2G), and a random chance of observing the L3-R3-L3-R3/L3-R3-L3-L3 organization (47.7 ± 0.2% [±SD]). Note that to obtain a probability less than 50%, cells would have to actively prevent the two
Fig. 1. Anucleate cell formation in the absence of MukB is biased toward mother cell newer poles (A) Schematic of mother machine microfluidics device and representative cells in a channel. ΔmukB cells contain ori1 and ter3 FROS markers and a segmentation marker (gray). A nongrowing anucleate cell lacking FROS markers is indicated. (Scale bar, 1 μm.) (B) ori1 and ter3 localization as a function of cell length in ΔmukB (21,057 cells) and WT cells (287,900 cells). Sample numbers with different cell lengths were normalized by the maximum value in each vertical bin. (C) Number of ori1 foci prior to anucleate (one of the daughters is anucleate; 2,444 cells) and normal cell division in ΔmukB cells (10,468 cells) and in WT cells (22,224 cells). A two-sample t test was used between mean ori1 numbers prior to anucleate and normal division in ΔmukB (P value < 10^{-5}) and between normal division in ΔmukB and WT (P value 0.0037). (D) Percentage of anucleate cell divisions in ΔmukB (14,392 divisions) and WT (22,511 divisions) and the percentage of anucleate cells forming at a mother cell’s old and newer poles in ΔmukB (2,269 divisions). Data are from three repeats in ΔmukB and two repeats in WT in all analyses.

chromosomes from having the same orientation. ter3 migration pattern of ΔmukB cells showed a similar localization pattern to WT with even earlier migration to the cell center (SI Appendix, Fig. S2A), in contrast to the richer medium condition in Fig. 1. Our observations show that the absence of MukBEF causes the impairment of both the distinctive L-R chromosome organization prior to replication and the L-R-L-R organization after replication.

Meanwhile, ΔmatP cells exhibited chromosome locus localization patterns strikingly different from that of WT and ΔmukB cells (Fig. 2 C–E). The average distance between L3 and R3 was reduced twofold (Fig. 2F), which also prevented L3 and R3 from being directed into opposite cell halves (65.7 ± 0.8% [±SD]; Fig. 2G). Concomitantly, it also led to the L3 and R3 loci being preferentially localized closer to the cell center than in WT cells, where L3 and R3 localize toward the cell poles (Fig. 2F). The ter3 pattern was less precise, lacked the stepwise migration pattern to cell center, and exhibited earlier segregation of the locus (SI Appendix, Fig. S2A), in agreement with previous studies (18). Despite these substantial perturbations, the L3-R3-L3-R3 organization was retained in ΔmatP cells prior to cell division (80.2 ± 1.9% [±SD]), indicating that other processes must act in determining the observed organization.

To determine if the absence of MatP influences chromosome organization–segregation over generations, we followed WT and ΔmatP cells using time-lapse imaging. We observed that ΔmatP cells retained the L3-R3-L3-R3 (or R3-L3-R3-L3) orientation in only 32.2 ± 4.6% (±SD) of daughter cells, while most WT cells retained the orientation (91.4 ± 5.2% [±SD]; Fig. 2H and SI Appendix, Fig. S2 B–E and Movies S2 and S3). We next assessed when the marker flipping occurs during the cell cycle. Prior to the duplication of L3 and R3 loci, ΔmatP cells flipped the orientation on average 0.78 ± 0.02 (±SD) times per cell cycle, compared to 0.08 ± 0.01 (±SD) of WT cells (Fig. 2I and J), while the propensity to flip orientation increased with replication–segregation progression, reaching twofold just before the duplication of the L3 and R3 loci (Fig. 2F; for WT see SI Appendix, Fig. S2K). Therefore, locus flipping is not restricted to nonreplicating chromosomes. Once duplicated, the L3-R3-L3-R3 orientation (or R3-L3-R3-L3) was found to be stable until cell division in both WT and ΔmatP cells (99.7 ± 0.01% [±SD] and 93.8 ± 0.02% [±SD], respectively, SI Appendix, Fig. S2 G and H). The fraction of other configurations (L3-R3-R3-L3 and R3-L3-R3-R3) remained the same in ΔmatP and WT (SI Appendix, Fig. S2L), and ΔmatP daughter cells with flipped chromosome arms were initially born with the same orientation as in the mother cell (88.4 ± 2.8% [±SD]; SI Appendix, Fig. S2F).

Overall, most L3-R3-L3-R3 orientation flips to R3-L3-R3-L3 (and vice versa) arose as a consequence of L3-R3 to R3-L3 flips (and vice versa) prior to locus duplication, followed by locus replication–segregation.
Fig. 2. MukBEF and MatP action generates and propagates L-R chromosome organization in E. coli. (A) E. coli chromosome circular map with ori1, ter3, L3, and R3 loci. MukBEF complexes are displaced from the 800-kbp ter region by matS bound MatP. (B) Representative images of WT, ΔmukB, and ΔmatP cells with ori1 and ter3 or L3 and R3 FROS markers. Note an atypical R3-L3-L3-R3 configuration in WT (white arrow) in comparison to the standard L3-R3-L3-R3. (Scale bars, 1 μm.) L3 and R3 localizations (C) and ori1 localizations (D) along the long cell axis as a function of cell length in WT (L3-R3 57,509 cells and ori1 42,612 cells), ΔmukB (L3-R3 27,984 cells and ori1 54,820 cells), and ΔmatP (L3-R3 46,679 cells and ori1 51,350 cells). Sample numbers with different cell lengths were normalized by the maximum value in each vertical bin. Cells are oriented to place L3 more toward the negative pole (toward figure bottom) or, in the ori1 data, ter3 is oriented more toward the negative pole (SI Appendix, Fig. S2). White lines denote cell borders. (E) Overlay of ori1 and L3-R3 localization data in WT and ΔmatP from C and D. (F) Distance between L3 and R3 markers in WT (47,376 cells), ΔmukB (15,615 cells), and ΔmatP (41,625 cells) in single L3 and R3 focus cells. Mean and dispersion (SD) between experiments are shown above each distribution. Error bars denote SD of the cell population. * and ** denote two-sample t test of L3-R3 distances between WT and ΔmukB (P value 0.0081) and WT and ΔmatP (P value 5 x 10^-6), respectively. (G, Left) Percentage of cells with L3 and R3 in opposite cell halves in single L3 and R3 focus cells (WT 47,376 cells, ΔmukB 15,615 cells, and ΔmatP 41,625 cells). (Right) Percentage of cells with L3-R3-L3-R3 (or R3-L3-R3-L3) configuration (versus L3-R3-L3-R3 or R3-L3-R3-L3) in double L3 and R3 focus cells (WT 10,352 cells, ΔmukB 2,535 cells, and ΔmatP 6,297 cells). The dashed horizontal line indicates random localization, assuming that each sister cell inherits a complete chromosome. (Scale bars, 1 μm.) (H) Percentage of cells retaining L3-R3-L3-R3 orientation (versus flipping to R3-L3-R3-L3) from a mother cell to a daughter cell in WT (859 pairs) and ΔmatP (1,034 pairs). (Scale bars, 1 μm.) (I, Top) Number of L3-R3 flipping events (±SD) to R3-L3 (or vice versa) during a cell cycle in WT (3,059 cells) and ΔmatP cells (4,102 cells) (SI Appendix, Fig. S2 I and J). (Bottom) Probability of L3-R3 flipping to R3-L3 (or vice versa) (blue) and L3-R3-L3-R3 flipping to R3-L3-L3-R3 (or vice versa) (orange) as a function of cell length in ΔmatP (10,362 cells). The flipping probability was normalized by the number of cells in each bin. The gray box indicates the replication period as a function of cell size from Fig. 3. The red vertical line indicates the average cell length at locus duplication (±SD between experiments). Data are from three repeats in all analyses.
Lagging Strand Segregation to the Cell Center, Marked by DnaN, Is Dependent on MukBEF. Translational symmetry of sister chromosomes arises at least in part from the symmetric segregation of lagging strands toward midcell during DNA replication (and leading strands toward the cell poles), as shown using an elegant genetic system (21). Here, we sought directly to visualize the positioning of lagging strands in WT, mukBΔ, and mukBΔmatPΔ cells.

During replication, ~40 DNA-bound β2-clamps, which ensure DNA polymerase III processivity, have a ~3 μm residence time on DNA before they are unloaded (33). The DNA-bound clamps are expected to accumulate largely on the lagging strand and its template because new clamps are loaded during synthesis of each Okazaki fragment (Fig. 3A). We reasoned that since β2-clamps could potentially cover >100 kb of newly replicated lagging strand DNA, they could serve as a marker to monitor lagging strand segregation. As a reference for the localization of replication forks, we imaged fluorescent DNA polymerase III ε-subunits (DnaQ) in the same cells. Indeed, while DnaQ foci were more spread toward cell poles, as previously described (34), DnaQ foci localized closer to the cell center; consistent with the lagging strands being directed to midcell (Fig. 3 B and C). By measuring the distance from each DnaQ focus to the closest DnaN focus in each cell, we found that 41.2 ± 5% (± SD) of DnaQ foci do not colocalize (i.e., further apart than the diffusion limit dictates, ~300 nm) with DnaN foci during replication (Fig. 3D). The differential location of DnaN and replication forks was confirmed by the measurement of the distances from replicative helicase (DnaB) foci to their closest DnaN focus (47.1 ± 6.1% ± SD) not colocalizing) (SI Appendix, Fig. S3 A–C). Since DnaN and DnaQ colocalize during early and late replication, when sister replisomes are necessarily close together, we also analyzed the localization patterns for cells that are in the middle of the replication cycle (Fig. 3E), when independently tracking replication forks are more frequently spatially separate. The pattern of DnaQ foci that did not colocalize with DnaN foci (SI Appendix, Fig. S3F) underlines the conclusion that spatially separate sister replisomes in opposite cell halves have a different cellular location from DnaN. Our results are consistent with the previous independent measurements of DnaQ and DnaN localization and the observation that DnaN foci of sister replisomes often do not spatially separate (34–36). Here, we provide direct evidence that the replisome and β2-clamp complexes frequently do not colocalize during replication. Our visualization of the segregation of lagging strands during replication supports the previously shown symmetric segregation of leading strands toward the cell poles (21).

To analyze how MukBEF and MatP contribute to lagging strand segregation, we measured DnaN and DnaQ localization in ΔmatPΔ and ΔmukBΔ cells. The DnaN distribution in ΔmatPΔ cells was broader than in WT cells (Fig. 3 E and F), indicative of spatially less precise lagging strand segregation but still directed toward cell centers, as predicted by the L3-R3-L3-R3 organization. The DnaN distribution in midcycle ΔmatPΔ cells was more central than that of DnaN (50.8 ± 1.5% ± SD) colocalization with DnaN during replication; SI Appendix, Fig. S3E), most likely because of less separated chromosome arms, as shown by L3 and R3 markers (Fig. 2F). Both DnaQ and DnaN exhibited a broader distribution at shorter cell lengths (Fig. 3F), presumably because of a more random chromosome conformation (Fig. 2). ΔmukBΔ cells showed a distribution of DnaN and DnaQ localizations toward cell poles, with almost identical patterns for both markers (Fig. 3G and 1 and 2 focus heatmaps in SI Appendix, Fig. S3 H and I). The results show that lagging strands and their templates cannot be directed to cell centers in a timely manner in the absence of MukBEF function, a result consistent with impaired L3-R3-L3-R3-L3-R3 organization in ΔmukBΔ cells (Fig. 2G). By measuring the distance from each DnaQ focus to the closest DnaN focus, we found that lagging strands did not leave the vicinity of the replisome during the DnaN dwell time on chromosomes of ~3 min (78.4 ± 0.5% ±SD colocalization; SI Appendix, Fig. S3G). We hypothesize that this is a consequence of delayed decatenation by TopoIV in the absence of MukBEF (37), since lagging strand templates can only be segregated from the leading strands once decatenation has occurred. Note that the generation times of WT, ΔmatPΔ, and ΔmukBΔ cells are comparable (18), with replication initiating and completing in the same cell cycle in most cells of all three strains (SI Appendix, Fig. S3F). This is in agreement with previous “runout” experiments (18), in which a fraction of ΔmukB populations having four chromosomes likely result from replication in the two-chromosome sister cells of an anucleate cell division.

We also examined the dynamin-like protein CrfC (aka YjdA), which has been proposed to bind β2-clamps and tether the nascent strands of sister chromosomes together (38). However, upon the deletion of crfC, we observed no changes to DnaN localization along the long cell axis (SI Appendix, Fig. S3 K and L) or any decrease in the frequency of the L3-R3-L3-R3 configuration (SI Appendix, Fig. S3M). This result indicates that CrfC is not necessary for WT chromosome conformation and segregation.

Ancestral DNA Strands Are Preferentially Retained at Older Cell Poles. Previously, it has been hypothesized that a symmetrical segregation of lagging strands to the cell center of sister chromosomes and, in consequence, the older template DNA strand (here referred as the ancestral strand since they are inherited from the grandmother generation or earlier) is not randomly segregated to daughter cells over subsequent generations but preferentially retained in the daughter with the older cell pole (discussed in ref. 20). Cell division generates two new cell poles at the division septum, while the other ends of the daughter cells are the older poles that were created in an earlier division. To address this theory directly, we developed a pulse-chase assay that allowed us to visualize the relative age of DNA strands between sister chromosomes and relate their position to the age of the pole without the need for cell synchronization or tracking (Fig. 4A).

The assay is comprised of the pulse labeling of newly replicated DNA and identifying the relative pole age by chemo-receptor accumulation at cell poles. The newly synthesized DNA was labeled by a 15 min EdU (5-ethynyl-2-deoxyuridine) pulse, after which cells were washed and allowed to grow for 3 h (generation time ~150 min). To avoid EdU-mediated growth defects, thymidine was added to the medium to outcompete EdU. We observed no detrimental effects on growth rate or cell size from the low concentration of EdU used in the pulse (SI Appendix, Fig. S4). After the growth period, most cells have completed the following round of replication, resulting in only one of the two sister chromosomes containing the EdU label (Fig. 4A). Cells were then fixed; EdU was visualized by click chemistry using Alexa 488 azide and nucleoids labeled by DAPI (Fig. 4B). As a result, in cells with completely replicated and segregated nucleoids (D-period), the chromosome with the newer template strand will be fluorescently labeled, while the one with the ancestral strand is not (Fig. 4C and SI Appendix, Fig. S4D).

To identify the older cell pole, we exploited the fact that the serine chemoreceptor, Tsr, accumulates approximately linearly with time at the cell poles (39). Hence, the older pole can be distinguished from the new pole by a higher quantity of fluorescently labeled Tsr. Because imaging the Tsr-GFP fusion used before (39) was incompatible with EdU staining, we used a functional HaloTag fusion of the endogenous tsr gene labeled with synthetic tetramethylrhodamine (TMR) dye. This allowed us to determine if the older strand chromosome was segregated toward the older or newer pole in each cell (Fig. 4C). In a control experiment, we confirmed that the intensity of Tsr-mYpet foci was higher at the older pole in 99.2 ± 0.5% ± SD) of cells (Fig. 4D).

We observed that 71.3 ± 3.9% ±SD of WT cells contained EdU foci in the chromosome closer to the new pole (Fig. 4E). Because EdU was incorporated into the new template strand,
Fig. 3. DnaN visualizes the lagging strands during replication. (A) Schematic of the accumulation of β2-clamps (DnaN) on the lagging strand during replication (33). The DNA polymerase β-subunit (DnaQ) marks the location of the replisome. (B) Representative images of WT cells with fluorescently labeled DnaN and DnaQ. (Scale bars, 1 μm.) (C) DnaQ and DnaN localization in WT cells as a function of cell length (37,720 cells). White lines denote cell borders. Shaded areas denote intermediate cell lengths for localization data in E. (D) Distance from a DnaQ focus to the closest DnaN focus. DnaQ and DnaN colocalize in 58.8 ± 5% (±SD) of focus pairs (38,855 pairs), as defined by a distance threshold according to the diffraction limit (300 nm, red lines). Inset shows the same data as a cumulative distribution. The same data as in C. (E) DnaQ or DnaN localization with intermediate cell lengths (3.3 to 3.7 μm) in WT (DnaN 7,104 and DnaQ 8,006 spots), ΔmukB (DnaN 11,925 and DnaQ 8,025 spots), and ΔmukB (DnaN 5060, DnaQ 4205 spots) cells (see C, F, and G). Full width at half maximum of the distribution in WT: DnaN 0.67 ± 0.06 μm and DnaQ 1.67 ± 0.08 μm and in ΔmukB: DnaN 1.85 ± 0.04 μm and DnaQ 1.14 ± 0.14 μm (±SD). Gray areas denote cell poles. DnaQ and DnaN localization in ΔmukB cells (51,956 cells) (F) and in ΔmukB cells (22,902 cells) (G) as a function of cell length. White lines denote cell borders. Data are from three repeats in all analyses.

this indicates that the ancestral strand is preferentially retained at the older pole. The result deviates significantly from random retention, in which the older pole would have a 50% chance of inheriting either strand (binomial two-tailed test P value < 10−5). We also compared the dispersion (SD) of our data to a binomial distribution with different sample sizes to estimate the reliability of our experiment (SI Appendix, Fig. S4E). We found excellent agreement, showing that our measurements are robust for the given sample size, with no additional noise sources, and increasing data sample size would give diminishing returns.

How is ancestral strand retention related to chromosome organization? To address this question, we tested the contributions of MukBEF and MatP to ancestral strand retention. Upon deletion of matP, we observed a random segregation of the ancestral strand (48.5 ± 3.8% [±SD]; Fig. 4E), demonstrating that functional MukBEF is required for ancestral strand retention at older poles. Deletion of matP also abolished the preferential segregation of the ancestral strand (46.2 ± 1.1% [±SD]; Fig. 4E). While MatP has not been implicated in early chromosome segregation, when the segregation pattern(s) emerge, the influence of MatP on MukBEF action is crucial as it prevents chromosome arm flipping (Fig. 2H), which would disrupt the association of the ancestral strand with the older pole. MatP-matS also interacts with the divisome through ZapB, and this interaction has been proposed to partially anchor ter to the inner cell membrane (40). This interaction could plausibly contribute to the ancestral strand retention by anchoring the chromosome and thereby preventing chromosome rotation. However, upon replacing the native matP with a nondivisome-interacting matPΔC20 mutant or deleting zapB, we did not observe any difference to WT with regard to ancestral strand retention (71.8 ± 3.1% and 71.8 ± 1.0%, respectively [±SD]; Fig. 4E). This confirms that the loss of ancestral strand retention in ΔmatP cells is related to the proposed chromosome rotation, measured by L3 and R3 flipping along the longitudinal cell axis over generations.

Finally, since MukBEF and MatP have coevolved with a group of proteins (including Dam and SeqA) that are related to Dam DNA methyltransferase activity (41), we tested the influence of these proteins on the retention of the ancestral strand. The delayed methylation of adenines in the sequence GATC transiently distinguishes the parental and newly synthesised strands after replication. Prior to Dam methylation, SeqA binds to hemimethylated
GATC sites, negatively regulating replication initiation and possibly contributing to chromosome segregation (reviewed in ref. 42). Deletion of either *dam* or *seqA* did not influence ancestral strand retention at older poles (69.9 ± 1.5% and 67.6 ± 3.3%, respectively [±SD]; Fig. 4E), indicating that GATC methylation patterns do not affect the observed asymmetry and, consequently, overall L-R chromosome organization.

**Discussion**

Our results demonstrate how MukBEF directs the nucleoid organization and nonrandom segregation of sister chromosomes in *E. coli*. The rigorous analyses of genetic locus positioning in relation to the localization of MukBEF, replisomes, and newly replicated lagging strand, in a range of WT and mutant strains, provide insights into the molecular mechanisms underlying *E. coli* chromosome organization and segregation and complement previous studies that have quantified the nucleoid dynamics in mechanical terms (17, 31, 43, 44). The major observations are the following: 1) anucleate cells arise at the new pole in *ΔmukB* cells, and frequently in cells that have unsegregated oriC at the older pole cell half; 2) MukBEF and MatP have distinct roles in the generation and propagation of translationally symmetric chromosome organization over generations; 3) DNA-bound β2-processivity clamps, which mark lagging strands and localize to the cell center, dependent on MukBEF action and independent of replisome location; and 4) ancestral (immortal) DNA strands are preferentially retained in the sister cell with the older cell pole, dependent on MukBEF and MatP. We address how we interpret these observations below and present a model (Fig. 5) that integrates our conclusions and proposals with those of previous reports, thereby providing a conceptual foundation for understanding how nucleoid conformation and dynamics shape subcellular organization.

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Fig. 4. Visualization of ancestral DNA strand retention in *E. coli*. (A) Ancestral DNA strand propagation shown following an EdU pulse and the subsequent growth. After the second round of replication, only one of the chromosomes inherits the EdU label. Note that only a part of the chromosome is labeled with EdU. The 15-min EdU pulse and growth period are also shown relative to a schematic of cell-cycle stages (B, C and D periods; generation time growth. After the second round of replication, only one of the chromosomes inherits the EdU label. Note that only a part of the chromosome is labeled with EdU. 

**Discussion**

Our results demonstrate how MukBEF directs the nucleoid organization and nonrandom segregation of sister chromosomes in *E. coli*. The rigorous analyses of genetic locus positioning in relation to the localization of MukBEF, replisomes, and newly replicated lagging strand, in a range of WT and mutant strains, provide insights into the molecular mechanisms underlying *E. coli* chromosome organization and segregation and complement previous studies that have quantified the nucleoid dynamics in mechanical terms (17, 31, 43, 44). The major observations are the following: 1) anucleate cells arise at the new pole in *ΔmukB* cells, and frequently in cells that have unsegregated oriC at the older pole cell half; 2) MukBEF and MatP have distinct roles in the generation and propagation of translationally symmetric chromosome organization over generations; 3) DNA-bound β2-processivity clamps, which mark lagging strands and localize to the cell center, dependent on MukBEF action and independent of replisome location; and 4) ancestral (immortal) DNA strands are preferentially retained in the sister cell with the older cell pole, dependent on MukBEF and MatP. We address how we interpret these observations below and present a model (Fig. 5) that integrates our conclusions and proposals with those of previous reports, thereby providing a conceptual foundation for understanding how nucleoid conformation and dynamics shape subcellular organization.
**E. coli Chromosome Organization.** The *E. coli* chromosome is organized into a nucleoid filament with chromosome loci positioned linearly along the longitudinal cell axis outside of the ter region (12, 32, 45, 46). Stiff nucleoid “bundles” that are radially confined by cell dimensions and exhibit a contour length of the scale of cell dimensions were characterized in live-imaging studies of *E. coli* (31). Bundles were also identified in cells with increased volume, which allowed the visualization of nonreplicating, toroidal chromosomes (47). Previous attempts to explain the precise chromosome loci positioning (e.g., by a randomly oriented polymer or transcription factor–mediated DNA loops) failed to provide the molecular requirements for maintaining chromosome conformation and orientation inside a cell (10, 43). We propose that the lengthwise compaction of the chromosome by a linear MukBEF axial core (17) can explain the formation of the nucleoid bundles (31) and the linear nature of chromosome loci positioning along the longitudinal cell axis outside of ter (Fig. 5). Linear MukBEF axial cores arise by the matS-MatP–mediated depletion of MukBEF from ter, which breaks the symmetry of otherwise circular chromosomes (17, 18). Continuous axial cores were observed in cells in which MukBEF occupancy on the chromosome was modestly increased (~3.3-fold), while cells with WT cells exhibited more granular, but indistinguishable, MukBEF localization inside a cell (17). Theoretical studies have demonstrated that lengthwise compaction of the chromosome forms stiff bundles that promotes individualization of chromosome arms through excluded volume interactions and by the maximization of conformational entropy (48, 49). In the absence of MatP, MukBEF cannot be depleted from ter, and cells are unable to direct chromosome arms to opposite cell halves efficiently (Fig. 2 F and G).

We propose that this is because the circular MukBEF axial cores of ΔmatP cells bring chromosome arms closer together than in WT cells (Fig. 5) (17). A less compacted and more “relaxed” ter region in WT cells might be required for efficient chromosome segregation during fast growth, since ∆matP cells exhibit more frequent anucleate cell production than MatP+ cells (50).

The frequent L3-R3 locus flipping in ΔmatP cells likely reflects global changes in the nucleoid, rather than local, locus-specific effects because genetic loci have predictable localization patterns in cells that recapitulate the physical and high-throughput chromosome conformation capture (Hi-C) contact maps of the chromosome (12, 32, 45, 46, 51). Furthermore, Hi-C analysis showed that deletion of MatP only affects chromosome organization in the ter region ~300 kb away from L3 and R3 markers, and large excursions of chromosome loci were found to be rare outside replication–segregation of a specific locus (52). We therefore propose that the observed locus flipping can be explained by whole-chromosome rotation that displaces chromosomal loci along the longitudinal cell axis. Any intermediate value between 90° and a complete 180° rotation would flip the L3-R3 locus orientation (Fig. 5C). In our model, in WT cells, a linear chromosome bundle [as opposed to uniform, circular chromosome bundle in ΔmatP cells (47)] restricts chromosome rotation, thereby explaining how L3-R3-L3-R3 (or R3-L3-R3-L3) configuration can be stably propagated over generations without obvious membrane anchoring (Fig. 5). Other mechanism(s) may additionally contribute to the maintenance of chromosome orientation.

A nondivisome-interacting MatP mutant displayed similar segregation behavior to WT, ruling out divisome tethering as a possible mechanism (see Fig. 4; ancestral strand retention would be lost if L3-R3 flipping would occur like in ΔmatP cells). A chromosome membrane-tethering strategy is generally found in organisms in which MukBEF has been replaced by Smc-ScpAB complexes and which carry a parABS segregation system [e.g., through PopZ in *C. crescentus* (53), HubP in *Vibrio cholera* (54), and RacA/DivIVA in *sporangium of B. subtilis* (55, 56)]. Membrane anchoring typically uses ParB bound to oriC-proximal parS sites as an intermediary. Intriguingly, some bacteria, such as *V. cholera* or *P. aeruginosa*, not only encode MukBEF/SmcBEF but also specify a parABS system (9, 57). Whether organisms that encode MukBEF orthologs but not typical Smc-ScpAB complexes, and which lack ParABS systems, generally have life cycles that encompass overlapping replication cycles, similar to *E. coli*, remains to be determined.

In the absence of MukBEF, chromosome loci outside of the ter region were found to be generally more randomly localized (Fig. 2), in support of the hypothesis that MukBEF action positions the chromosome inside a cell through extensive intranucleoid interactions (51). The mislocalization of oriC toward older cell poles in ΔmukB cells may contribute to anucleate cell formation, since sister oriC need to move further apart than those in WT cells. An earlier analysis of locus positioning in ΔmukB cells led to the proposal that the impaired chromosome organization is frequently accompanied by the chromosome arms being aligned together along the long cell axis (16), an organization reminiscent of the situation in WT *C. crescentus* (3).
Sister Chromosome Replication and Segregation. A connection between translation symmetry of sister chromosome (L-R-L-R) and asymmetrical segregation of leading/lagging strands has been previously proposed (20, 21). Consistent with this, we observed the accumulation of β2-clamps, present primarily on lagging strands, toward cell centers of replicating cells, when compared to both DNA polymerase III and helicase localization (Figs. 3 and 5). Differential positioning of the replisome and β2-clamps in WT cells also resolves the conundrum that emerged from studies that favored a model of a single-replication “factory” containing two replisomes at the cell center, based on clamp labeling (36). Our results support the model of independent tracking of the two often spatially separated replisomes in cells undergoing a single round of replication (34, 58), although segregation forces along with the reorganization of parental and newly replicated DNA leads to the frequent movement of sister replisomes toward the cell center.

A similar behavior was observed in ΔmatP cells, but in the absence of MukB, β2-clamps localized toward cell poles, coincident with replisomes. This shows that symmetric lagging strand segregation to the cell center determines the L-R-L-R segregation pattern of sister chromosomes, while a nearly random pattern of daughter chromosomes was observed in the absence of MukB. The presence of the L-R-L-R pattern in both WT and ΔmatP cells rules out chromosome orientation or chromosome arm separation as a requirement for establishing this pattern. We also refuted a previous hypothesis that a dynamin-like protein YjdA (aka CrfC) contributes to the symmetric lagging strand segregation by linking together the β2-clamp–loaded, nascent DNA strands (38). We hypothesize that MukBEF could plausibly differentiate between leading and lagging strands, leaving lagging strands less compacted (Fig. 5).

Our results also lead us to propose that the lifetime of individual, chromosome-associated clamps [estimated to be ~3 min (33)] must be longer than the sister chromosome cohesion time for chromosomal regions outside of oriC and ter (estimated to be ~14 min and ~9 min, respectively) (18, 59). Precise measurements of cohesion times have been refractory to accurate experimental determination. Cohesion time between newly replicated sisters is at least partly determined by the time required for TopoIV to remove replicative catenanes (18, 57, 59), although tethering of ter to the divisome through MatP–ZapB interactions may and influence cohesion time in this region (60). MukBEF promotes TopoIV catalysis (18, 37). Therefore, delayed oriC segregation in ΔmukB cells, which was particularly evident in the relatively fast growing cells in the microfluidics experiments, could reflect impaired decatenation, since the decatenase TopoIV is no longer recruited by MukBEF to oriC-proximal regions. Indeed, modest overexpression of TopoIV led to a reduction in the cohesion time of newly replicated oriC from ~14 min to ~5 min in Muk+ cells (59). Delayed ori decatenation of ΔmukB cells may contribute to nonviability under fast growth conditions, while slow growth conditions allow sufficient time for chromosome decatenation and segregation in most cells. Nevertheless, the relative contributions of oriC misslocalization and delayed decatenation remain unknown.

Ancestral Strand Retention at the Older Pole. We have directly shown that the older template (“ancestral”) DNA strand is preferentially segregated to the older pole cell in E. coli. This nonrandom segregation is determined by the translational symmetry of the sister chromosomes (L-R-L-R), prior to cell division, and efficient maintenance of chromosome orientation over generations by MukBEF and MatP. However, as E. coli lacks the properties of cell differentiation, development, and regeneration of a multicellular organism, it is not clear why it has evolved a chromosome organization that nonrandomly segregates the ancestral strand to daughter cells. While E. coli cells can grow with a constant rate for hundreds of generations (30), the death rate was found to increase with replicative cell age, which was attributed to the growth-independent accumulation of protein damage (30). Increasing cellular maintenance processes through the general stress response reduced the death rate, while its absence increased it (61). Old pole cells have been shown to exhibit a diminished growth rate following the accumulation of cellular damage and misfolded protein aggregates (62, 63). The older pole also accumulates more membrane proteins (e.g., chemoreceptors and efflux pumps) than the new pole; this can significantly contribute to cell survival in challenging environments (39, 64). For example, the main multidrug efflux pump of E. coli, AcrAB-ToIC, displays increased efflux activity in older pole cells compared to new pole cells, giving a growth advantage under subinhibitory antibiotic concentrations and possibly against other toxic compounds (64). AcrAB-ToIC pump activity is also required for acquiring a resistance gene from mobile genetic elements in the presence of antibiotics (65). Finally, a common epigenetic mechanism to regulate phase variation in bacteria involves the formation of DNA methylation patterns by proteins binding near a hemimethylated GATC site and blocking methylation (e.g., pap or foo, clp, and pep systems), which all encode pil (66). Preferential retention of the ancestral strand could potentially allow the old pole cell to maintain the previous methylated state. In the end, ancestral strand retention could simply be an evolutionary by-product of maintaining the L-R chromosome organization over replication–division cycles. Since ancestral strand retention occurs in only ~70% of older-pole cells, this gives opportunities for selection in fluctuating or harmful environments, independent of whether older or newer pole cells thrive better.

Materials and Methods
Detailed information of all experimental procedures is provided in SI Appendix. In brief, E. coli K12 AB1157 derived strains (SI Appendix, Table S1) were created using standard molecular biology and genetics techniques. Cells were grown in M9 0.2% glycerol minimal medium supplemented with five amino acids and thiamine at 30 °C, except for the microfluidics experiments in which cells were grown in M9 0.2% glucose supplemented with MEM amino acids and thiamine at 37 °C. For microscopy, cells were diluted 1,000-fold from an overnight culture, grown to an A600 of ~0.1, and spotted on an M9 glycerol agar plate with 1% agarose pad on a microscope slide or placed inside the microfluidics device, as in ref. 29. Inside the microfluidics device, cells were imaged every 5 min for >18 h. Agarose pad time lapses for chromosome arm flipping were imaged every 10 min for 3 h at 30 °C. Imaging was performed on a Nikon Ti-E microscope equipped with perfect focus system, 100x numerical aperture 1.4 oil objective,scCMOS camera (Hamamatsu Orca Flash 4), temperature chamber (Oklabs), and light-emitting diode excitation source (Lumencor SpectraX). For EdU experiments, cells were labeled with 10 μM EdU for 15 min, washed, introduced to fresh media containing 60 μg/mL thymidine, and allowed to grow for 3 h with shaking. Following this, cells were fixed, permeabilized, and a click chemistry reaction (Thermo Fisher Scientific, #C10337) was conducted using Alexa 488 azide. Finally, Tsr HaloTag was labeled with TMR HaloTag ligand, as in ref. 67, and nucleoids were visualized by DAPI. All image analysis and cell tracking were performed using SuperSegger (68) in MATLAB (Mathworks). Further data analysis and statistics were also performed in MATLAB.

Data Availability. All study data are included in the article and/or supporting information.

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