Post-replicative pairing of sister ter regions in Escherichia coli involves multiple activities of MatP

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The ter region of the bacterial chromosome, where replication terminates, is the last to be segregated before cell division in Escherichia coli. Delayed segregation is controlled by the MatP protein, which binds to specific sites (matS) within ter, and interacts with other proteins such as ZapB. Here, we investigate the role of MatP by combining short-time mobility analyses of the ter locus with biochemical approaches. We find that ter mobility is similar to that of a non-ter locus, except when sister ter loci are paired after replication. This effect depends on MatP, the persistence of catenanes, and ZapB. We characterise MatP/DNA complexes and conclude that MatP binds DNA as a tetramer, but bridging matS sites in a DNA-rich environment remains infrequent. We propose that tetramerisation of MatP links matS sites with ZapB and/or with non-specific DNA to promote optimal pairing of sister ter regions until cell division.

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https://doi.org/10.1038/s41467-020-17606-6

NATURE COMMUNICATIONS | (2020) 11:3796 | https://doi.org/10.1038/s41467-020-17606-6 | www.nature.com/naturecommunications
Bacterial nucleoid structurisation is due to a variety of processes including DNA supercoiling, proteins and complexes working on the DNA (e.g., RNA polymerases), nucleoid-associated proteins and condensins (SMCs)\(^1\). These act together to shape the chromosomes in a dynamic structure while keeping DNA accessible to polymerases and repair proteins. Replication and segregation re-organise nucleoids on a large scale\(^2\). Whilst some details may vary, common to all bacteria is the bidirectional replication of the chromosome, starting at a unique origin and finishing in the opposite terminus region, which is the last to be segregated before cell division.

Macrodomains are large regions with specific cellular positioning that contain either the origin or terminus of replication\(^4\,^8\). In *Escherichia coli* (*E. coli*), the Ter macromdomain (ter) is very distinctive (Fig. 1a), spreading along 800 kb encompassing the replication terminus region\(^7\). It contains 23 matS sites that are recognised by the MatP protein\(^7\). MatP is known to play a key role in ter positioning and setting its segregation pattern: it keeps the sister ter regions paired near the divisome, at mid-cell, allowing their processing by the DNA-translocase FtsK\(^10\). Since FtsK activity is oriented by KOPS DNA motifs, segregation ends at the *dif* site, where final unlinking of sister chromosomes occurs\(^10,^11\).

How MatP achieves its functions is currently unclear. MatP forms dimers in the absence of DNA, and tetramers upon binding to matS-containing DNA\(^12\). This was proposed to pair matS sites, forming large chromosome loops, though they were not detected in contact maps of the chromosome\(^13\). MatP was also shown to interact with the divisome-associated ZapB protein\(^14\) and the condensin MukB\(^15\). A truncated variant of MatP (deletion of the last 20 residues), MatPA20, was reported unable to form tetramers\(^12\) nor to interact with ZapB\(^14\), yet retaining interaction with MukB\(^15\). MukBEF was reported to promote long-range interactions between chromosome loci, probably by forming loops, in a MatP-dependent manner\(^13,^16\). Consistent with this view, MatP and MatPA20 lower long-range interactions and/or promote short-range interactions inside ter\(^13,^17\) while excluding MukB from ter\(^15\). Since MukB interacts with TopoIV, its exclusion by MatP probably delays decatenation of sister ter, thus coupling their segregation with cell division\(^15\). Interaction of MatP with ZapB has been proposed to induce a positive control of divisome assembly around ter, (the Ter-linkage)\(^17,^18\). Mutation of zapB, as well as matPA20, alters the mid-cell positioning of MatP-bound sister ter and shortens the co-localisation times of ter loci\(^12,^14\).

Foci movements have also been recorded at different time scales, revealing important differences between chromosome regions. At long time scales, loci tracking captures their segregation dynamics\(^19\,^24\). Loci of *ter* localise accurately at mid-cell\(^19\), then separate when early divisome components have formed a complex at mid-cell. At short-time intervals, they sub-diffuse\(^1,^25\,^27\), reflecting constraints imposed by their environment\(^9,^13,^27\). A previous study showed that the mobility of loci varied depending on chromosomal localisation\(^27\), the *ter* loci being less mobile when located at mid-cell.

In this report, we investigate the role of MatP in constraining the mobility of a *ter* locus. Surprisingly, low-fluorescence foci of the *ter* locus are as mobile as those of an oriC-proximal locus, showing that the higher constraint of the *ter* locus is not an intrinsic property but depends on context. We further show that highly intense and poorly mobile foci form most often at the *ter* locus and depend on the presence of MatP, suggesting they contain pairs of unsegregated sister loci. This effect depends on MatP, its 20 C-terminal residues and ZapB to different levels. We characterise MatP/DNA complexes and conclude that while MatP binds DNA as a tetramer, it rarely forms specific DNA loops by bridging *matS* sites in a DNA-rich environment, suggesting that the tetramers play a different role.

**Results**

**Monitoring chromosome loci mobility in vivo.** To monitor the mobility of chromosome loci, we used strains carrying a *parS* site inserted on the chromosome and producing cognate ParB-GFP proteins\(^29\) (Fig. 1a). We recorded the position of foci every 0.5 s during 20 s (Fig. 1b), then extracted the mean squared displacements (MSD) from these trajectories. An example of 30 MSD for Ter4 is shown in Fig. 1c.

We first used the P1 *parS* site and ParB protein to tag loci in the *ori* and *ter* regions, and we reproduced published results for Ori2 and Ter3\(^27\) (Fig. 1e). However, this P1-derived system has been reported to increase post-replicative cohesion of tagged loci\(^12,^28\). We thus tested another set of strains, with loci tagged with *parS*-pMT1 and producing a ParB-pMT1-GFP\(^28\). The comparison revealed important differences: (1) The number of cells with a single focus decreased and those with two foci increased when using the pMT1-derived system (Fig. 1d); (2) The MSDs obtained with the pMT1 system were larger than with the P1 system (Fig. 1e); (3) The difference in MSDs between the *ori* and *ter* loci were largely reduced when using the pMT1-derived system (Fig. 1e); (4) For a same intensity, a remarkable drop of mobility was observed for Ter loci labelled with *parS*-P1\(^27\). The P1-derived system thus not only delays *ter* segregation, but creates aggregates of proteins that result in brighter foci (Supplementary Fig. 1) with very low mobility, biasing the results obtained. We chose to proceed with the pMT1-derived system in this work.

The mobility of a *ter* locus depends on foci intensity. We next analysed the fate of foci formed at Ori2 and Ter4. Foci populations were binned into categories depending on their number per cell and their localisation, for which we defined two categories: (M) mid-cell (0–1/6th cell length from the cell centre) and (R) rest of the cell (1/6th–1/2). Consistent with previous reports\(^29\,^31\), segregated foci of Ori2 preferentially localised at the quarter positions, and near mid-cell for single foci. Also consistent with previous reports, Ter4 foci were preferentially located at mid-cell (Supplementary Fig. 2), unsegregated foci being closer from mid-cell than segregated ones.

Foci intensity varied between loci. Ori2 foci followed a sharp distribution centred around 500 AU (Fig. 2a). In contrast, Ter4 foci followed a wider distribution with more high-intensity foci. For moderately intense foci (below 1000 AU), we observed a monotonous decrease of mobility when intensity increased (Fig. 1f). Strikingly, Ori2 and Ter4 foci had equivalent MSDs at corresponding intensities. The low mobility of *ter* loci is thus not an intrinsic property of *ter* but depends on the intensity of the foci (Fig. 1f). At intensities above 1000 AU, foci mobility no longer varied in a monotonous way with increasing intensity and was clearly different between Ori2 and Ter4. From this observation, we defined two categories of foci: foci of low intensity, hereafter called FL, below 1000 AU, and foci of high intensity, hereafter called FH, above 1000 AU. FH were rare at Ori2 (2%) but rather frequent at Ter4 (30%) (Fig. 2b). Double FH of Ter4 were rare and tended to position like double FL.

Calibrating the number of GFP molecules in foci (from the increase of variance in intensity along time) gave an estimated mean of 33 GFP molecules for Ter4 and Ori2 FL, whereas the mean for Ter4 FH was 70 GFP (Supplementary Fig. 3 and “Methods”).

**FL and FH show different dynamics.** We analysed the trajectory of foci using four parameters characterising the nature of movement (“Methods”\(^32\)): (1) The anomalous exponent (α) is computed from the MSD behaviour for small increments. It
indicates the nature of the locus motion. \( \alpha = 1 \) describes normal diffusion, \( \alpha < 1 \) is sub-diffusive (constrained) and \( \alpha > 1 \) is super-diffusive (directed) movement. (2) The length of confinement (\( L_c \)) is the standard deviation (SD) of the locus position with respect to its mean averaged over time. This estimates the apparent radius of the volume explored by a finite trajectory. (3) The diffusion coefficient (\( D_c \)) reflects the second-order statistical properties of a trajectory, and accounts for local crowding that may vary along the trajectory. (4) The effective spring coefficient (\( K_c \)) represents an external force acting on a locus. It is modelled as a spring force applied on a single monomer belonging to a polymer. This force affects the entire polymer motion and can be recovered from the first-order moment statistics of single locus trajectories.

Considering only FL (Fig. 2d), the value of each parameter was poorly dependent of their position, suggesting that loci properties do not change significantly during the cell cycle. Ori2 loci had a low value of \( \alpha \) (0.16), whereas Ter4 showed a slightly higher \( \alpha \) (0.2, \( p = 3.8 \times 10^{-13} \)), suggesting that the local condensation is slightly higher in ori than in ter, according to the RCL model\(^{22-24}\). The length of confinement for both loci was small (0.084 and 0.081 \( \mu m \) for Ori2 and Ter4, respectively), revealing loci are confined in small regions, which size depends slightly on chromosomal or cellular location. The diffusion coefficient was higher for Ori2 than for Ter4 (3.5 \( \times 10^{-3} \) and 2.9 \( \times 10^{-3} \) \( \mu m^2/s \), respectively, \( p = 2.7 \times 10^{-22} \)), showing that despite a greater condensation, Ori2 is freer to diffuse than Ter4. Finally, the spring coefficient revealed equivalent forces tethering both Ori2 and Ter4 (331 and 319 \( k_B T \mu m^{-2} \)). In cells harbouring two foci, 2FL showed the same behaviour as 1FL (Supplementary Fig. 4a).

Ter4 FH behaved differently than FL (Fig. 2d). Ter4 FH \( \alpha \) was close to the FL value (0.22 for FH and 0.2 for FL, \( p = 7 \times 10^{-5} \)) and remained the same for both cell positions (\( \rho = 0.5 \)), suggesting a monotonous condensation of the ter region. However, \( L_c \) was lower than for FL (\( p = 10^{-125} \)) and lower at mid-cell than in the rest of the cell (\( p = 8 \times 10^{-2} \)). This suggests Ter4 FH are more confined than FL and more confined when at mid-cell. \( D_c \) showed the same trends as \( L_c \), showing that Ter4 FH are diffusing less than FL (\( p = 10^{-200} \)) and less when in the mid-cell area (\( p = 5 \times 10^{-5} \)). These changes are consistent with a doubling in the force applied to Ter4 FH when at mid-cell (620 \( k_B T \mu m^{-2} \) for FH and 331 \( k_B T \mu m^{-2} \) for FL, \( p = 10^{-130} \)).

Ter4 FH are thus, compared to FL: brighter, submitted to twice the force, more confined, less diffusive and preferentially located at mid-cell. A straightforward hypothesis is that they mostly contain sister loci held together in post-replicative cohesion. This is consistent with the extended cohesion period reported for sister ter regions\(^{21,29,30}\), even when the pMT1 system was used\(^{10}\). This also explains the higher percentage of FH for Ter4 compared to Ori2. It follows that most FL contain single copies of loci. Assuming this hypothesis, a strong constrain is applied to ter loci only when they are in post-replicative cohesion. The rest of the time, their dynamics is much more similar to the one of other chromosome regions than previously thought.
MatP is required for FH formation and maintenance in ter. We next deleted matP from our strains and observed the fate of Ter4 foci. The strongest effect was on FH. Cells with one FH decreased drastically (from 27 to 8%; Fig. 3a), whereas cells with one FL decreased moderately (from 58 to 48%). Consistently, cells with two foci increased and mostly contained FL. This increase in two-foci cells is consistent with MatP acting to keep sister ter DNA. We observed a clear effect on Ter4 FH (Fig. 3b, p = 10^{-8}, p = 1.9 \times 10^{-12}, p = 7.6 \times 10^{-11}, respectively). These values are close to the ones obtained for Ori2 FH (Supplementary Data 2, p = 0.75, p = 4 \times 10^{-3}, p = 0.56 for L_c, D_c and K_c). In addition, FH at mid-cell were not different anymore from the ones located in the rest of the cell (Fig. 3b, p = 0.6, p = 0.4, p = 0.5 for L_c, D_c and K_c). We conclude that MatP is required both for the high number of FH Ter4 and for their specific constraint at mid-cell. A slight but significant difference in mobility and confinement between FH and FL in the Δ(matP) strain persisted (Fig. 3c; p = 4 \times 10^{-17}, p = 2 \times 10^{-33} and p = 4 \times 10^{-14} for L_c, D_c and K_c). Remaining FH may be rare single loci with high fluorescence intensity, which should reduce their mobility. However, a fraction of remaining FH certainly contains foci present on chromosome dimers, which may pair during FtsK/Xer resolving process, since this is MatP-independent. Interestingly, deletion of matP had only a slight effect on α of Ter4 FH (p = 0.02) and none on FL (p = 0.06), suggesting that MatP only marginally influences the local condensation of ter DNA.

Deleting matP also had a small but significant effect on Ter4 FL dynamics (Fig. 3c). They were less confined and more mobile in the Δ(matP) strain (higher L_c (p = 3 \times 10^{-7}) and D_c (p = 6 \times 10^{-7}), lower K_c (p = 7 \times 10^{-10})). This appears as a general effect, as equivalent variations were observed with Ori2 (Supplementary Fig. 5b).

Different activities of MatP are required for FH formation. We then used two mutants: a deletion of zapB, coding for the divisome-associated protein interacting with MatP, and matPΔ20, coding for a version of MatP deleted of the last 20 C-terminal residues, defective for both interaction with ZapB and tetramerisation. In these two mutants, MatP still binds matS (ref. 12, and below) and interacts with MukB.

Both Δ(zapB) and matPΔ20 mutants showed a phenotype intermediate between the wild-type and Δ(matP) strains considering...
Only one MatP activity is mediated by TopoIV control. Since MatP was proposed to control the removal of catenanes by TopoIV \(^{15}\), we assayed the effect of a moderate overproduction of both TopoIV subunits. This was previously reported to shorten the post-replicative co-localisation period of non-ter loci by premature resolution of precatenanes \(^{37}\). Overproduction of TopoIV increased the portion of cells with a single FL, suggesting it somehow modifies the cell cycle, delaying replication and/or postponing cell division (Fig. 4). As expected, the number of Ori2 FH decreased (from 1.3 to 0.5%), consistent again with most FH being paired loci. Strikingly, this effect was not observed at Ter4 in the wild-type strain but was clearly observed in both the

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**Fig. 3** Ter4 foci are more mobile in matP mutants. a Proportion of each type of focus in Ter4 mutant strains. The four parameters \(a\), \(L_c\), \(D_c\), and \(K_c\) were calculated for 1FH Ter4 foci (b), and 1FL Ter4 foci (c). Wild-type (pink) and mutant backgrounds (\(\Delta\text{matP}\), blue; \(\text{matP}\Delta20\), green; \(\Delta\text{zapB}\), orange) are presented as in Fig. 2d. To help comparing the values, a line has been drawn at the value for the foci at mid-cell in the wt background, the value indicated in pink, and the outliers at high values have been cut off. Box plots show the median of the distribution, the 25th and 75th percentiles of the samples (respectively the bottom and top of the box), the lowest and top values within a range of 1.5 times the interquartile range (dotted lines), and outliers of these as crosses. The notches display the variability of the median; non-overlapping notches between samples indicate different value of the medians at a 5% significance level. b Ter4 wt, \(n=1374\) for M, \(n=412\) for R; Ter4\(\Delta\text{matP}\), \(n=258\) for M, \(n=115\) for R; Ter4\(\Delta\text{matP}\Delta20\), \(n=968\) for M, \(n=267\) for R; Ter4\(\Delta\text{zapB}\), \(n=475\) for M, \(n=174\) for R. c Ter4 wt, \(n=2744\) for M, \(n=1064\) for R; Ter4\(\Delta\text{matP}\), \(n=1057\) for M, \(n=754\) for R; Ter4\(\Delta\text{matP}\Delta20\), \(n=1805\) for M, \(n=918\) for R; Ter4\(\Delta\text{zapB}\), \(n=1598\) for M, \(n=786\) for R. Source data are provided as a Source Data file.

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**Fig. 4** An increased amount of TopoIV decreases the number of FH in mutant strains. Proportion of each type of focus for Ori2 (O2) or Ter4 (T4) strains. The TopoIV was slightly overproduced in strains marked as TopoIV+.

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**Fig. 5 MatP can bridge DNA.** a. Scheme of the pull-down experiment. A biotinylated DNA (DNAb), containing 0, 1 or 2 matS, is attached to a streptavidin-covered, magnetic bead. Free DNA (DNAf), containing 0, 1 or 2 matS, is added to the reaction, alongside a competitor (40 bp, non-specific, double-stranded DNA). Different concentrations of MatP are added to the mix. The reactions are pulled-down a first time, rinsed with buffer, eluted with 0.1% SDS, which denatures MatP but not the streptavidin, and pulled-down again. DNAf is recovered if MatP has induced bridging. Those are then loaded on an agarose gel. b–d Examples of gels obtained with both DNAb and DNAf containing 2 matS (b), no matS (c) or 1 matS on DNAf (d). Concentration of MatP is from left to right: 0, 0.1, 0.25, 0.5, 1 and 2 μM, with an additional reaction with 4 μM for (c). The percentage of recovered DNAf is shown below each lane; lane C shows the initial amount of DNAf (20 ng). Quantitation can vary from gel to gel but the trend is the same within three independent experiments. The type of DNA observed on the gel is indicated on the left of the gel (f for DNAf). Molecular weight markers are indicated on the other side, in kbp.

MatP bridges DNA molecules in vitro. As we assume FH contain paired loci dependent on MatP, we attempted to characterise DNA bridging by MatP. We designed an assay based on DNA pull-down (Fig. 5a). A biotinylated DNA molecule containing 0–2 matS was bound to a streptavidin-covered magnetic bead (DNAb). This complex was mixed with a free DNA (DNAf) containing 0 or 2 matS. The mix was then incubated with MatP, washed, pulled-down with a magnet and eluted with a low concentration of SDS. The amount of DNAf recovered represents the capacity of MatP to bridge the two DNA molecules. When they both contained two matS (Fig. 5b), 18% of DNAf were recovered with the lowest concentration of MatP used (0.1 μM), increasing to 63% at the highest concentration (2 μM). When repeating the same experiment with DNA that did not contain matS, DNAf was recovered with lower efficiency (Fig. 5c). When only one DNA contained a matS, the amount of DNAf recovered was intermediate (Fig. 5d) and Supplementary Fig. 6). We conclude that MatP is able to bridge independent DNA molecules containing or devoid of matS. The presence of matS stimulates this activity and/or stabilises the complexes formed.

MatP bridging activity involves non-specific DNA binding. To better describe MatP bridging activity, we used a high-throughput tethered particle motion (TPM) set-up. This tracks beads attached at one end of a DNA molecule while the other extremity is tethered to a coverslip (Fig. 6a). The amplitude of motion at equilibrium of the bead (Aeq) directly depends on the apparent length of the DNA. We used DNA containing 0, 1 or 2 matS separated by 1207 bp. The traces (example Fig. 6b) are plotted as densities of probability of their Aeq and fitted to Gaussian distributions (Fig. 6c). Without protein we observed a single population centred on 250 nm (Fig. 6c, *). Adding MatP resulted in the displacement of the whole population toward shorter Aeq, independently of the DNA used. This corresponds to an apparent shortening of around 30 nm (Fig. 6c, **). This moderate decrease in Aeq cannot correspond to MatP-induced DNA looping between the matS sites, because it is independent on matS. Moreover, the shortening predicted from bridging two matS would be around 100 nm. An equivalent moderate decrease in Aeq was previously observed using another site-specific, DNA-binding protein in the same set-up and was attributed to protein binding to a single site. Surprisingly, this moderate decrease in Aeq was not observed with MatPA20 (Fig. 6c). We verified that MatPA20 and MatP bound matS-containing DNA (Supplementary Fig. 7a). Tagged or untagged versions of MatPA20 bound a matS-containing DNA with equivalent efficiency as MatP. However, the mobility of the complexes was unexpected. Indeed, MatPA20-tag formed complexes migrating faster than MatP, despite being twice bigger (Fig. 7). Assuming MatPA20 binds DNA as a dimer, these observations suggest that MatP binds DNA as a tetramer. This explains the difference observed between MatP and MatPA20 in TPM experiments, suggesting the moderate decrease in Aeq is due to tetrameric MatP binding to a single matS, or to non-specific DNA, and changing DNA conformation.

A second population with shorter Aeq was obtained upon incubation of the 2-matS DNA with MatP (Fig. 6c, ***). Gaussian fitting of this population indicates that it was centred at 147 nm (±22), corresponding to the 100 nm shortening predicted for looping between two matS. Accordingly, this peak was neither observed when using DNA without matS nor when using MatPA20 (Fig. 6c). However, it was also detected upon incubation of the 1-matS DNA with MatP. This peak was centred on 153 nm (±23) and contained three times fewer events (13%) than with the 2-matS DNA (36%). The second peak formed with DNA containing one or two matS exhibited very similar centres, as if 1200 bp was the most favourable inter-matS distance for looping by MatP. We next repeated the experiment using a 2-matS DNA with non-specific DNA as a competitor. The moderate decrease in Aeq was readily observed (Supplementary Fig. 8a, **), but the peak corresponding to a greater shortening was not, showing that pairing of distant DNA loci by MatP is

matPA20 and Δ(zapB) strains. There, the rate of Ter4 FH dropped to the one measured in the Δ(matP) strain (about 4%). We conclude that post-replicative co-localisation between sister ter loci results from at least two different mechanisms involving MatP: one depends on the last 20 C-terminal residues of MatP and on ZapB and is not affected by increased levels of TopoIV, the second one depends on an increased level of TopoIV, suggesting a MatP-dependent catenane persistence.
DNA loci in TPM. This activity depends on the 20 C-terminal residues of MatP, is stimulated by the presence of matS but is highly dynamic, and strongly sensitive to the presence of non-specific DNA.

This prompted us to analyse in more details the MatP/matS complexes. Using two DNA probes carrying a single matS site and labelled with different fluorophores, we performed an EMSA experiment showing the interaction between a 41 bp DNA fragment containing matS (MW\textsubscript{theo} = 27 kDa) and 3 \mu M of MatP (MW\textsubscript{theo} = 21 kDa), his-tagged MatP\Delta20 (MW\textsubscript{theo} = 32 kDa), or untagged MatP\Delta20 (MW\textsubscript{theo} = 18 kDa). Based on the theoretical molecular weight (MW\textsubscript{theo}) of the DNA molecule and of the different proteins used, we have estimated the theoretical molecular weight of the different protein-DNA complexes following three different hypotheses. Hypothesis 1 (Hyp1) proposes that MatP and MatP\Delta20 bind DNA as a dimer; hypothesis 2 (Hyp2) proposes that MatP and MatP\Delta20 bind DNA as a tetramer; hypothesis 3 (Hyp3) proposes that MatP binds DNA as a tetramer, whereas MatP\Delta20 binds DNA as a dimer. Note that only hypothesis 3 proposes theoretical molecular weights that are in accordance with EMSA results.

Taken together, these results show that MatP can pair distant DNA loci in TPM. This activity depends on the 20 C-terminal residues of MatP, is stimulated by the presence of matS but is highly dynamic, and strongly sensitive to the presence of non-specific DNA.

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sensitive to non-specific DNA competition. The analysis of the kinetics of loop formation (Supplementary Fig. 8b, c) showed that the presence of matS enhances the formation of loops by increasing the duration of long-lived looped events.

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Discussion

We combined different approaches to investigate the organisation of the ter region and the role of MatP. These results yield several new observations and unexpected conclusions: (1) ter loci are not intrinsically less mobile than ori loci; (2) a significant proportion of ter foci show a high fluorescence, preferentially when they are located at mid-cell, and are much less mobile; (3) the proportion of these foci and their low mobility depend on all described activities of MatP, specifying two separable processes, of which one depends on the persistence of catenanes, while the other one depends on interaction with ZapB; (4) MatP binds matS as a tetramer but this complex contains only one DNA fragment; (5) the bridging of matS sites can be observed in vitro but is efficiently competed by non-specific DNA. From these observations, we conclude that MatP constrains ter mobility only at a specific stage of the cell cycle, when replication has terminated and sister chromosomes are paired by their ter regions (D period of the cell cycle). MatP does so via at least two activities, only one depending on its C-terminal domain. This last activity certainly depends on tetramerisation, but not on bridging remote matS. Indeed, the pairing of matS can be observed in vitro but is readily challenged by non-specific DNA and is thus likely irrelevant in vivo.

Loci of the ter region have been reported to be less mobile than other regions\(^{9,12,14,21}\). However, these comparisons did not consider the context of the foci, particularly their intensity. Tracking foci over short-time intervals, focusing on local DNA constraints, has revealed that mobility depends on foci intensity and on their sub-cellular positioning\(^{25}\). Our data extend this observation and further show the importance of the labelling system. Indeed, using the less invasive pMT1-derived system, significant differences in mobility between an ori-proximal and a ter-proximal locus are observed only for the most intense foci (FH). Indeed, less intense foci (FL) of the ter and ori loci show the same decrease in mobility when intensity increases (Fig. 1). The trajectories of FL revealed slight but significant differences between ori and ter, including a decreased α anomalous coefficient, suggesting ori is more condensed than ter (Fig. 2). This may be consistent with recent images of the E. coli nucleoid showing a donut shape in round cells, in which the ter region appears less condensed than the rest of the chromosome in a MatP-dependent way\(^{42}\). Higher α coefficient may be linked to the MatP-mediated exclusion of MukBEF from ter\(^{13,15,16}\). However, the difference in α we measured is not MatP-dependent (compare Figs. 2 and 3), arguing against this hypothesis. Despite its higher condensation, the ori locus appears freer to diffuse than the ter locus, as shown by its higher diffusion coefficient (Fig. 2). This difference is only partly suppressed by a mutation of matP and better suppressed by a mutation of zapB (compare FL foci in Figs. 2 and 3). Since ZapB self-assembles into large structures and clusters around ter (in a MatP-dependent manner) and the divisome (in a MatP-independent manner)\(^{17,36,43,44}\), we suspect these clusters limit the diffusion of ter loci via an interaction with matS-bound MatP but also by a MatP-independent mechanism, yet to be described.
should lead to compaction of the ter region, which is argued against by the absence of anomalous component modification in $\Delta$matP (Fig. 3). In addition, chromatin immunoprecipitation of MatP did not reveal non-specific binding of MatP around matS sites. An attractive model would thus be that MatP tetramers specifically serve to bridge matS-containing DNA with ZapB, excluding other binding activity, so that the DNA-bridging activity of MatP would be observed only when ZapB is absent. While multiple findings point to the existence of a Ter/MatP/ZapB/ZapA/FtsZ complex in vivo, attempts to detect such an interaction in vitro (Supplementary Figs. 9 and 10) were unsuccessful. These negative results do not rule out the model and could be explained by the complexes being only transients or requiring other actors like ZapA and/or FtsZ.

Taken together, our data support the view that MatP mainly acts to pair sister ter regions until the onset of cell division but has little effect on their dynamics when unpaired. To do so, tetramers of MatP bind matS sites and act in at least two ways, which can be genetically separated. This is globally results in delaying decatenation by TopoIV until FtsK gets activated and segregate the ter regions by promoting both decatenation and dimer resolution at the diff site. 

Methods

Strains, media, plasmids. E. coli strains were all derived from MG1655 and provided by Espejo. Briefly, parS sequences were inserted at positions 4197685 bp for Ori2 locus (parS-P1 and parS-pMT1), 1395706 bp for Ter3 (parS-P1), and 1444252 bp for Ter4 (parS-pMT1) loci. Strains carrying a parS-P1 sequence were transformed with pAL2705, and strains carrying parS-pMT1 were transformed with pHCH2973.39 The $\Delta$matP and $\Delta$zapB deletions were transferred by P1 transduction from strains JW399 and JW3899 of the Keio collection. The matPΔ2020 strain was obtained from Boccard; the mutant gene was transduced into parS-pMT1 labelled strains. Ampicillin (50 µg/mL), kanamycin (25 µg/mL), and chloramphenicol (10 µg/mL) or spectinomycin (20 µg/mL) were added when necessary. To overexpress TopoV, strains were transformed with a pWX53 containing the spectinomycin resistance. The leakage from the arabinose promoter was sufficient to observe increased decatenation.

Microscopy measurements. Strains were grown at 30 °C in M9 broth (Difco) supplemented with complementary salts (MgSO₄ 2 mM, CaCl₂ 100 µM, tryptophan 4 µg mL⁻¹ and thymidine 5 µg mL⁻¹), glucose (0.4%) and CAA (0.1%) for 12 h, then diluted 2000× in fresh M9-g glucose (0.4%). At an OD600nm of 0.4%, strains were supplemented with complementary salts (Mg²⁺SO₄ 2 mM, CaCl₂ 100 µM), and chloramphenicol (10 µg/mL) or spectinomycin (20 µg/mL) were added when needed. To observe TopoV, strains were transformed with a pWX53 containing the spectinomycin resistance. The leakage from the arabinose promoter was sufficient to observe increased decatenation.

Calibration of the foci. In order to estimate the copy number of fluorophores, we implemented a custom intensity calibration method in MATLAB (R2018a), based on the principle described in ref. 31. The calibration method aims at estimating the ratio between the intensity of the foci and the number of GFP molecules by exploiting the intrinsic fluctuations of intensity generated by the random photobleaching process. The key idea is that the variance of the intensity drop depends on the number of emitting GFP molecules contained in the focus at the beginning of acquisition, i.e., a higher number of emitting GFP molecules corresponds to a smaller variance in the relative intensity loss (see below). We estimate the intensity/copy number ratio (calibration ratio) by binning the foci by initial intensity, and measuring at position $x_n$ and measured at position $x_0$ is represented by:

$$V = \frac{4}{3} \pi X$$

occupied by the trajectory and can be used on any kind of trajectory, as it does not require a plateau.

(4) The effective diffusion coefficient $D_e$ reflects the second-order statistical properties of a trajectory. This diffusion coefficient accounts for local crowding that may vary along the trajectory.

Detailed calculation. Given a focus with $n$ emitting molecules, the observed intensity will be:

$$I = n \tau$$

Here we aim at estimating the calibration ratio $\alpha$ from the fluctuations in the intensity drop due to photobleaching. At any given time $t$, the number of emitting GFP molecules in a focus with $n_0$ initial emitting molecules, is described by a binomial distribution with $n = n_0$ and $p = \alpha$ where $r$ is the bleaching time constant. The variance of the binomial distribution is given by:

$$\text{Var}(n_0) = n_0p(1 - p)$$

where $\Delta = n_0p(1 - p)$ and $\text{Var}(\Delta) = \alpha$ represents the number of bleached molecules, and $p$ the probability of bleaching until time $t$. Then the contribution of bleaching to the variance of the intensity drop is:

$$\text{Var}(\Delta) = \frac{1}{2} \alpha n_0(1 - p)$$

of \( L_g(1 - p) \) on a linear fit (with intercept constrained at 0) on several initial intensity bins (Supplementary Fig. 3c). \( p \) is estimated from the data through a fit on \( \Delta f \) as a function of \( \Delta t \) (Supplementary Fig. 3a). This also allows to estimate the bleaching time constant \( \tau_f \) that will be used in the following steps.

In order to estimate the contribution of bleaching to the total intensity variance, we model it as follows:

\[
\text{Var}(\Delta I)_{\text{total}} = \text{Var}(\Delta I) + \text{Var}(\Delta I)_{\text{bleach}} = \text{Var}(\Delta I) + \gamma I_0 + \gamma I_f + \eta \Delta f.
\]

(9)

where \( \gamma \) groups non-bleaching variance contributions such as shot noise, and is expected to be proportional to intensity.

Since we expect \( \text{Var}(\gamma) = \sigma^2 \) and \( \text{Var}(\Delta f) = \sigma^2 \eta \) we can substitute it in Eq. (9) and fit the following expression to the observed variance as a function of time (Supplementary Fig. 3b):

\[
\text{Var}(\Delta I)_{\text{total}} = \text{var}^{2} + \nu \Delta I_0 \left( \Delta I^2 + \Delta I^2 \right).
\]

(10)

The bleaching dependent part of variance (the second term of the expression) has a different functional dependence on time, and therefore can be disentangled from other sources of variance and used to estimate the calibration parameter through the above described linear fitting. This process also allowed to verify that the bleaching contribution is indeed the dominant contribution in the empirical variance. As an additional pre-processing step, we estimate and subtract background intensity by extrapolating the intensity at which no bleaching is observed (Supplementary Fig. 3a).

Electromobility shift assays (EMSA). The matS41 DNA (41 bp) was obtained by hybridising oligonucleotides matS41F and matS41R. matS237 DNA (237 bp) was obtained by PCR amplification using pEL3, a pl.N135 derivative containing the matS41 sequence (our lab collection matS site: GTGACAGGTGCAC), as a matrix and matS4 and matS3 as oligonucleotides. Binding reactions were done in buffer containing 10 mM Tris (pH 7.5), 125 mM NaCl, 2.5 mM MgCl2, 0.5 mM DTT, and 5% glycerol, in the presence of 1 µM of each DNA probe, 0.25 µg of poly(dI-dC) and different concentrations of indicated proteins (3 µM of proteins in Fig. 7 and Supplementary Fig. 9, and 3 µM and 6 µM in Supplementary Fig. 7) The reactions were incubated at 30 °C for 5 min and analysed on a non-native TGE-PAGE.

ZapB purification. A pET32 containing zapB was transformed into BL21DE3 cells. After 2 h induction with 1 mM IPTG, cells were centrifuged and pellets were resuspended in RB1 (20 mM Tris pH 7.5, 7.0 mM NaCl, 1 mM DTT, 5% glycerol and protease inhibitor (complete EDTA-Free, Roche)). Cells were lysed by sonication, centrifuged and resuspended in RB1 for a step of ultra-centrifugation (50,000 rpm for 90 min at 4 °C). The supernatant was loaded onto a heparin column (HiTrap FF column (GE Healthcare Life Sciences) and ZapB was eluted with an imidazol gradient (0–0.5 M). ZapB fractions were pooled, dialysed (20 mM Tris pH 7.5, 150 mM NaCl, 5 mM MgCl2) and loaded on a Superdex 200 (GE Healthcare Life Sciences). ZapB fractions were pooled and frozen.

Pull-down experiments with ZapB. This experiment is performed like other pull-down experiments but with different MatP concentrations (from 1 to 6 µM) with or without ZapB (from 2 to 20 µM). Each reaction was done in duplicate in order to be analysed by classical agarose gel electrophoresis or SDS-PAGE. For western-blot analysis, reactions were analysed by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad) using the Trans-Blot® Turbo™ transfer system (Bio-Rad). Membranes were blocked 1 h at RT or overnight at 4 °C in 5% non-fat powder milk in PBS containing 0.05% Tween 20. Antibodies were anti-His (1:1000) (Tanaka, #631210). Blots were developed by chemiluminescence using Clarity western ECL substrate (Bio-Rad), visualised with the ChemiDoc™ Touch imaging system (Bio-Rad) and analysed with Image Lab software (Bio-Rad).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Code availability

The codes used in this study to track the foci are available at https://github.com/ver228/bacteria-foci-tracker. The codes used to extract the parameters are accessible at http://biowetmetrics.org/.” “Nuclear Organisation” section.

Received: 23 September 2019; Accepted: 3 July 2020

Published online: 30 July 2020

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Acknowledgements
We would like to thank M. Cosentino Lagomarsino, A. Javer, J. Kotar, M. Panlilio, M. Wlodarski, for useful discussions and help with experimental setups. We are also grateful to F. Boccard and O. Espeli for the kind gifts of bacterial strains and plasmids. Research in the FC group is founded by the CNRS and the Université Paul Sabatier (UPS Toulouse). Research in PC lab was supported by HFSP (RGY0070/2014) and UKRI grant EP/T002778/1.

Author contributions
E.C., P.R., P.C. and F.C. conceived the project. E.C. performed the microscopy experiments. E.C., D.H., B.S., P.R., F.C. and P.C. discussed the microscopy results. E.C., C.T. and M.S. performed the T.P.M. experiments. E.C., M.S., P.R. and A.L. performed the biochemical experiments. T.B. designed and performed the foci calibrations. E.C., P.R., P.C. and F.C. wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-17606-6.

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Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

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