Supporting Information for

Role of MatP, ZapA, and ZapB in chromosomal organization and dynamics in Escherichia coli

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SI Text

**Determination of lateral and longitudinal widths of MatP foci from static images.** The widths were measured using a custom Matlab script. The analysis started by manually drawing a 5 pixels wide (540 nm) profile along the long axis of the cell, which corresponded approximately to the width of the nucleoid. To draw the profile reliably along the long axis of the cell through the MatP focus the image was zoomed in by a factor of about four. In this step we used an overlay image composed of a fluorescent image of the nucleoid and MatP focus to verify that the focus was located at the center of the nucleoid. Small shifts between different image planes were corrected before combining the two images in the overlay image. From the overlay image a line profile of the MatP-YPET or MatP-mCherry intensity line profile was extracted. The profile was fitted to a Gaussian yielding estimates for the longitudinal full width at half maximum (FWHM) and center coordinates of the MatP focus. Subsequently, the coordinates of a line perpendicular to the longitudinal profile were calculated. The calculated line passed through the intensity maximum of the Gaussian fit. The intensity profile along the perpendicular line was then fitted to another Gaussian. The FWHM from this fitting determined the lateral width of the MatP focus and the center coordinates of the focus in the image coordinate system. To determine the coordinates of the MatP focus relative to the cell coordinate system we segmented the cell based on its phase contrast image. Our segmentation algorithm was based on a publicly available PSICIC script (1). We modified this algorithm to automatically determine the intensity level that corresponds to the cell edges (2). The segmentation procedure yields the midline and outer cell contour. Examples of segmented cells are shown in Fig. 1A in the main text. The position of the focus was calculated relative to the midline of the cell to determine the offset of the focus. The same contours were also used to determine the cell length and width.

**Analysis of time-lapse sequences.** Analysis of time-lapse sequences started by extraction of MatP-YPET and HupA-mCherry intensity line profiles along the long axes of cell. The extraction of the profiles followed the procedure described in (2). The resulting profiles were plotted (as shown in Fig. 3C and 4C in the main text). From the inspection of these plots we determined the times when the constriction in nucleoid density distribution appeared and when the MatP focus centralized (Fig. 5D).
The extracted contours were also used to determine the location of the cell poles, nucleoid edges, MatP focal positions along the long axis of the cell, and the minima of chromosomal intensity distributions (Fig. 3D, 3E and 4D, 4E). The cell and nucleoid edges were defined based on inflection points of corresponding intensity profiles (phase and HupA-mCherry fluorescence, respectively). Positions of the MatP foci were determined by a Gaussian fit. The minima and modulation depth of chromosomal intensity distributions were also determined by Gaussian fitting. In the latter case, the intensity level around both sides of the minimum were used to determine the average intensity level of a given nucleoid. The intensity line profile was subtracted from the average value and the profile was fitted to a Gaussian. The height of the Gaussian fit was normalized by the average intensity level from the nucleoid to yield the modulation.

1. Guberman, J.M., Fay, A., Dworkin, J., Wingreen, N.S. and Gitai, Z. (2008) PSICIC: Noise and asymmetry in bacterial division revealed by computational image analysis at sub-pixel resolution. *PLoS Comput. Biol.*, 4, e1000233.

2. Bailey, M.W., Bissichia, P., Warren, B.T., Sherratt, D.J. and Männik, J. (2014) Evidence for divisome localization mechanisms independent of the Min system and SlmA in *Escherichia coli*. *PLoS Genet.*, 10, e1004504.
**Figure S1.** Verification of lateral elongation of the Ter focus using a different fluorescent label. A) Images of two representative cells where the MatP-mCherry labeled Ter region is localized at the center of the nucleoid. From left to right are fluorescent images of MatP-mCherry, DAPI labelled nucleoid, and a composite image where the two fluorescent images are overlaid with a phase contrast image. Scale bars are 2 μm. B) Widths of MatP-mCherry foci along the long and short axes of cells. FWHM$_{\text{lateral}}$ = 0.628±0.088 μm and FWHM$_{\text{longitudinal}}$ = 0.523±0.097 μm (N=123). C) Lateral width vs normalized offset of the focus from the cell center along short axes of cell Cell radius is used for normalization of the offset. Solid line is a linear fit to the data (FWHM$_{\text{lateral}}$ [μm] = 0.67-0.18*(normalized offset)).
Figure S2. A) Lateral widths of MatP-YPET foci as a function of cell length in WT cells. Data are from the same measurement as shown in Fig. 1B and C in the main text. Solid lines represent a linear fit $-0.003 \cdot (\text{Cell Length}) + 0.62$, Pearson $R = -0.005$.

Figure S3. Projection of MatP focus to microscope image plane for different cell orientations. Top row: cross-sections of a cell in two orientations that have been rotated by 90 degrees. MatP (small green circles) is assumed to be attached to the divisome in the vicinity of the cell membrane (black circle) along a stretch of the divisome. The red lines correspond to DNA. Bottom row: corresponding microscope projections. When MatP attachment sites are parallel to the image plane then the microscopic image shows an elongated line (left) at the center of the cell. When MatP attachment sites are perpendicular to the image plane, then the microscope image shows a dot at the cell border (right).
Figure S4. Images of representative cells of ΔzapB, ΔzapA and matPΔC strains. From left to right are fluorescent images of MatP-YPET, HupA-mCherry, and a composite image where the two fluorescent images are overlaid with a phase contrast image. Scale bars are 2 μm.
**Figure S5.** Widths of the Ter foci in longitudinal and lateral directions for $\Delta$zapA, $\Delta$zapB, and matP$\Delta$C strains. One of the three replicate measurements is shown.
Figure S6. Normalized depth of the minimum in the intensity distribution of a nucleoid label at the center of the nucleoid (modulation) as a function of time for individual cells. A) Data for the wild type cell shown in Fig. 3A-D. B) Data for the matPΔC cell shown in Fig. 4A-D. Solid line in panel A is fit to an exponential function. Horizontal dashed lines indicate times of cell division. C) Individual modulation curves that have been used to calculate the average modulation curve for wild type cells. The corresponding average curve is shown in the main text on panel 3E. D) The same for matPΔC cells. The corresponding average curve is shown in the main text on panel 4E.
Figure S7. Time-averaged distribution of chromosomal density in the presence of external force that pushes chromosomal masses towards the ends of the cylinder. A) Replicated fraction $f=0.83$. External force of an approximately constant magnitude is applied to the two arms of the chromosome pushing them into two opposite directions. As a result of this force, chromosomal mass accumulates against the ends of the cylindrical boundary, but a local minimum at the center of the chromosome distribution can still be seen. Here we do not account for decrease in diameter of chromosomal beads that can occur in the presence of the force. B) Two fully replicated chromosomes ($f=1.0$) without any force applied. A minimum at the center of the nucleoid is seen. The distributions of chromosomal masses in two individual chromosomes are also indicated. C) Two fully replicated chromosomes with an external force applied. The applied force is of the same form as in panel A.
Figure S8. Radius of gyration for the Ter region along the long axis of the cell ($R_g$) with and without cross-linking effects for different replicated fractions ($f$). Radius of gyration has been normalized by the bead radius ($R_{\text{bead}}$).

Figure S9. Time-averaged distribution of the Ter region and the whole chromosome in a model where MatP cross-linking effects have been included. Replicated fraction $f=0.83$. 

Table S1. List of strains used in the experiments.

| Strain | Genotype |
|--------|----------|
| RH3    | MG1655
        | ΔhupA::hupA-mCherry-frt
        | ΔmatP::matP-YPet-frt-kan-frt |
| RH8    | MG1655
        | ΔzapB::frt-cm-frt
        | ΔhupA::hupA-mCherry-frt
        | ΔmatP::matP-YPet-frt |
| RH16   | MG1655
        | ΔzapA::frt-kan-frt
        | ΔhupA::hupA-mCherry-frt
        | ΔmatP::matP-YPet-frt |
| JMAH6  | MG1655
        | ΔhupA::hupA-mCherry-frt
        | matP C terminale 20aa::YPET-frt-kanR-frt |
| WD2    | MG1655
        | ΔmatP::matP-mCherry-frt-kan-frt
        | ΔzipA::Plac-zipA-gfp-amp |