

Division site selection linked to inherited cell surface wave troughs in mycobacteria

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1 Cell division is tightly controlled in space and time to maintain cell size and ploidy within narrow bounds. In bacteria, the canonical Minicell (Min) and nucleoid occlusion (Noc) systems together ensure that division is restricted to midcell after completion of chromosome segregation¹. It is unknown how division site selection is controlled in bacteria that lack homologues of the Min and Noc proteins, including mycobacteria responsible for tuberculosis and other chronic infections². Here, we use correlated optical and atomic force microscopy^{3,4} to demonstrate that morphological landmarks (waveform troughs) on the undulating surface of mycobacterial cells correspond to future sites of cell division. Newborn cells inherit wave troughs from the (grand)mother cell and ultimately divide at the centre-most wave trough, making these morphological features the earliest known landmark of future division sites. In cells lacking the chromosome partitioning (Par) system, missegregation of chromosomes is accompanied by asymmetric cell division at off-centre wave troughs, resulting in the formation of anucleate cells. These results demonstrate that inherited morphological landmarks and chromosome positioning together restrict mycobacterial division to the midcell position.

Q2 Atomic force microscopy (AFM) has been used previously for static^{5,6} or short-term time-lapse⁷ imaging of mycobacteria, primarily to study the impact of antibiotics and antimicrobial peptides on nanoscale features of the mycobacterial cell surface. Here, we use long-term time-lapse AFM to track cell growth and division over multiple generations in *Mycobacterium smegmatis*, a non-pathogenic relative of *Mycobacterium tuberculosis* (Fig. 1a, Supplementary Videos 1 and 2 and Supplementary Figs 1 and 2). Unexpectedly, we found that the cell surface undulates along the long axis (Fig. 1b,c) in a roughly repeating waveform pattern with an average wavelength of $\sim 1.8 \mu\text{m}$ (Supplementary Fig. 3). These morphological features are too small in amplitude ($\sim 100 \text{ nm}$ from wave crest to wave trough) to resolve by conventional optical microscopy and they are morphologically distinct from the previously described ‘division scars’⁸ (Supplementary Fig. 4, last panel, black arrow). Cell elongation is accompanied by an increase in wave trough number, as cells, on average, are born with three wave troughs and divide with four wave troughs after elongating by $2 \mu\text{m}$ (Supplementary Table 1). In contrast, the distance between wave troughs does not scale with increasing cell length. Cells filamented with ciprofloxacin exhibit a greater number of wave troughs as a function of increased cell length (Supplementary Fig. 6). Conversely, blocking cell elongation with isoniazid (Supplementary Fig. 2a) prevents the formation of new wave troughs (Supplementary Fig. 7). While the undulating

surface morphology is maintained in isoniazid-treated cells, height increases along the cell length (Supplementary Figs 2b and 8), possibly due to sustained metabolic activity.

In time-lapse series, we found that centrally located wave troughs correspond to future sites of cell division (Fig. 1c and Supplementary Figs 9–11). The centre-most wave trough is localized, on average, at 56% of the cell length relative to the old cell pole, ranging from 49% to 62% (25th to 75th percentiles, respectively). Remarkably, wave troughs that mark future division sites are already present at birth—they form near the cell poles in the mother, grandmother or great-grandmother cell and are passed on to the daughter cells at division (Fig. 2a,b, Supplementary Fig. 11 and Supplementary Table 1). On average, division at a wave trough occurs 1.3 generations after the trough is first established (Fig. 2b and Supplementary Table 1), which corresponds to $\sim 4 \text{ h}$ for cells growing with an average interdivision time of $\sim 3 \text{ h}$ (Fig. 2c). Cell elongation gradually shifts the position of wave troughs towards the cell centre (Fig. 2a, Supplementary Video 2 and Supplementary Figs 4 and 11)⁹. Inherited wave troughs localize to positions near the midcell $\sim 150 \text{ min}$ before cell cleavage. In cells inheriting multiple wave troughs, the amount of growth from each cell pole determines which wave trough is located closest to the midcell and becomes the division site. Depletion of RipA, a hydrolase essential for cleavage¹⁰, results in chains of non-separated daughter cells; cells located internal to the chain (with no free ends) do not elongate and do not form new wave troughs, although they may still form septa within pre-existing wave troughs (Supplementary Video 3 and Supplementary Fig. 5).

In a microscope that combines optical (fluorescence) and low-noise AFM-based imaging⁴, time-lapse imaging of single cells revealed a sequence of morphological and molecular events leading up to cell division. Formation of the FtsZ contractile ring at midcell is thought to be the earliest event specifying the cell division site in rod-shaped bacteria¹¹. In cells expressing FtsZ tagged with green fluorescent protein (GFP), we found that the FtsZ ring forms within a pre-existing wave trough near midcell (Fig. 3a). In cells growing with an average interdivision time of 190 min, formation of the pre-divisional wave trough precedes formation of the FtsZ ring by 120 min on average. Cells filamented with mitomycin C exhibit the formation of multiple FtsZ rings at multiple wave troughs (Supplementary Fig. 12).

Formation of the FtsZ-GFP ring near midcell (Fig. 3a and Supplementary Video 4) is followed $\sim 30 \text{ min}$ later by the appearance of a co-localized ‘pre-cleavage furrow’ ($\sim 50 \text{ nm}$ wide and $\sim 10 \text{ nm}$ deep) in the AFM image, a distinctive topological feature that is too small to detect by optical microscopy (Fig. 3a, black arrows). Q3 This feature might correspond to the previously reported ‘cell wall

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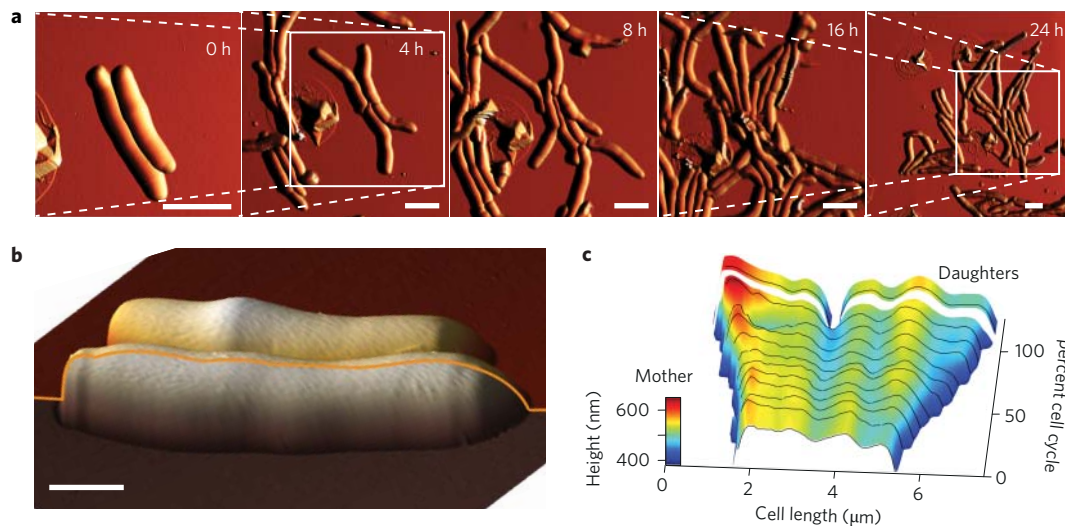


Figure 1 | Mycobacterial cells divide at cell surface wave troughs. **a**, Time series of three-dimensional AFM height images overlaid with AFM peak force error images for wild-type *M. smegmatis*. Scale bar, 3 μm . **b**, Mycobacterial surface topology. Yellow trace of the cell profile, highlighting the undulating mycobacterial surface morphology. Scale bar, 1 μm . **c**, Kymograph of the cell surface height of a representative cell from birth to division, showing that division occurs within the centre-most wave trough. In **b** and **c**, images are representative of $n = 270$.

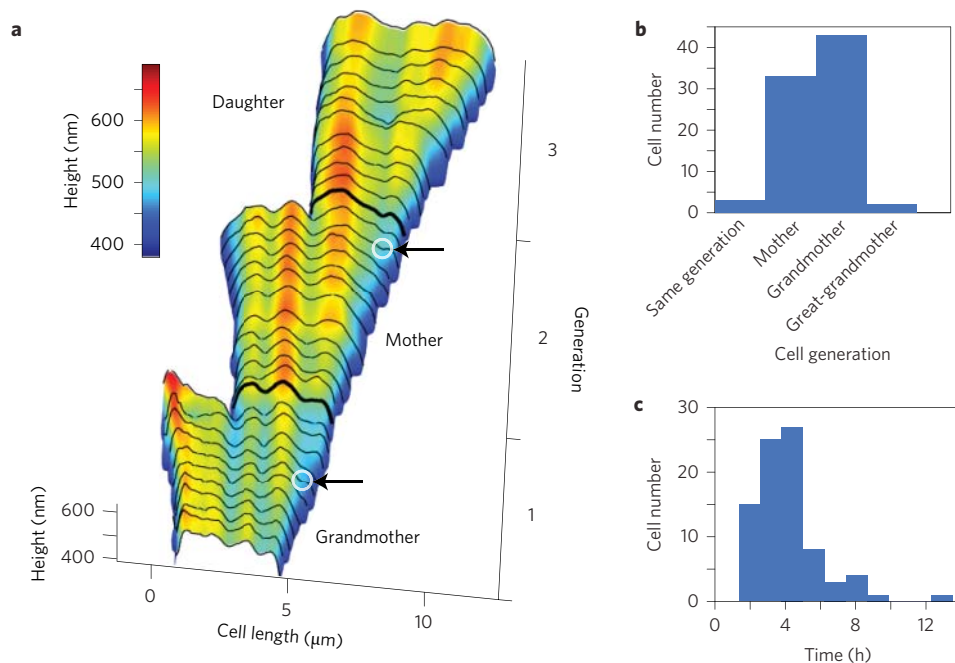


Figure 2 | Wave troughs are inherited from the (grand)mother cell. **a**, Kymograph of cell heights of one cell lineage over three consecutive generations (bottom to top). A wave trough formed in the grandmother cell (arrow) becomes the division site in the mother cell. Similarly, a wave trough formed in the mother cell (arrow) becomes the division site in the daughter cell. The kymograph represents 234 trios of related cells (grandmother–mother–daughter) from 18 unrelated cell lineages. **b**, Distribution of generations from wave trough formation to cell cleavage. The wave trough where division occurs in the daughter cell (generation 0) is usually formed in the mother cell (generation 1), grandmother cell (generation 2) or great-grandmother cell (generation 3). $n = 82$ cells. **c**, Distribution of time intervals from wave trough formation to cell cleavage. $n = 82$ cells.

1 contractile ring' in *Mycobacterium* sp. JLS, although the latter
 2 has been described as a cell surface protrusion rather than an
 3 indentation⁷. The pre-cleavage furrow appears at around the same
 4 time as the early stages of septum formation, which we visualized
 5 by staining the cell membrane with the fluorescent dye FM4-64
 6 (Fig. 3b, Supplementary Fig. 13 and Supplementary Videos 5 and 6).
 7 These events precede cytokinesis by ~ 20 min in cells expressing
 8 the cytokinetic marker Wag31-GFP (Fig. 3c and Supplementary

9 Video 7). Cytokinesis is followed by a lag period of ~ 40 min
 10 before physical cleavage of the sibling cells, signalled by an abrupt
 11 deepening of the pre-cleavage furrow to ~ 100 nm (Fig. 3 and
 12 Supplementary Video 7).

13 Most mycobacterial cells inherit multiple wave troughs at birth,
 14 yet only the centre-most wave trough is ultimately selected as the
 15 division site. We asked whether off-centre wave troughs could
 16 function as alternative sites of cell division in cells lacking the

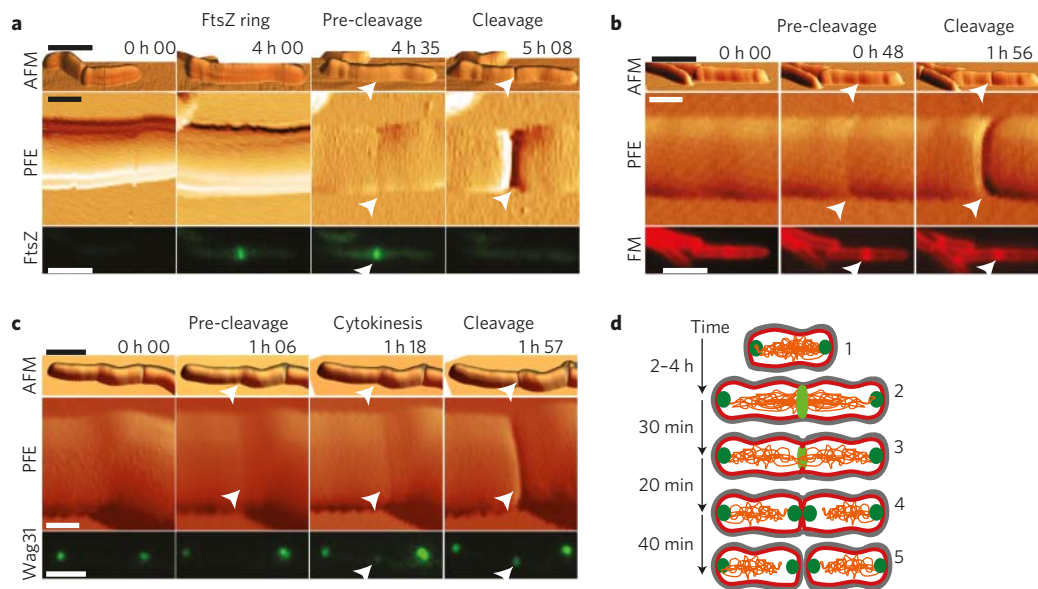


Figure 3 | Sequence of events from cell birth to cell division. Cells were imaged by correlated AFM (upper and middle panels) and fluorescence microscopy (bottom panels). Upper panels: three-dimensional representations of AFM topography images. Middle panels: AFM peak force error, which highlights the appearance of the pre-cleavage furrow and cleavage (white arrowheads). Numbers indicate time elapsed since birth. Scale bars (upper and bottom), 3 μm ; (middle), 500 nm. Images are representative of $n > 10$. **a**, Cells expressing FtsZ-GFP. The FtsZ ring co-localizes with the pre-cleavage furrow (images are representative of $n = 17$). **b**, Cells with FM4-64-stained plasma membrane. Septum invagination co-localizes with the pre-cleavage furrow (images are representative of $n = 15$). **c**, Cells expressing the cytokinesis marker Wag31-GFP. Cytokinesis is completed before cell cleavage (images are representative of $n = 50$). **d**, Schematic of the sequence of events culminating in cell division. At birth, Wag31-GFP (dark green) localizes exclusively at the cell poles (1). FtsZ-GFP (light green) forms a circumferential ring within the central wave trough (2–3). A pre-cleavage furrow then appears (indentation in the outer grey layer of the cell surface) and co-localizes with the FtsZ ring (3). Septum formation then proceeds and culminates in cytokinesis, which is marked by the appearance of a Wag31-GFP ring that co-localizes with the pre-cleavage furrow (4). Cell cleavage results in physical separation of the newborn sibling cells (5).

1 ParB chromosome-partitioning protein. Consistent with recent
2 studies^{12–14}, we found that ΔparB cells frequently undergo asym-
3 metric cell divisions (Fig. 4a) within off-centre wave troughs
4 (Fig. 4b,c and Supplementary Fig. 14). Divisions occurring at
5 off-centre wave troughs are skewed towards the old or new cell
6 pole (15% or 25%, respectively), with the remainder of divisions
7 (60%) occurring at the centre-most wave trough. Divisions in
8 ΔparB cells often occur in newly formed wave troughs
9 (Supplementary Fig. 15).

10 Time-lapse fluorescence microscopy revealed that midcell
11 divisions in ΔparB cells are associated with normal chromosome
12 partitioning (Fig. 4d). We never observed divisions occurring at a
13 local DNA maximum (Supplementary Figs 16 and 17). These
14 results suggest that chromosomes might play a negative regulatory
15 role in determining which wave trough is selected as the division
16 site. Cells treated with the DNA gyrase inhibitor ciprofloxacin
17 form elongated filaments with multiple wave troughs (Fig. 4e, first
18 time point). Appearance of a pre-cleavage furrow in a filamented
19 cell corresponds spatially to a local DNA minimum (Fig. 4e,
20 arrows; Supplementary Fig. 6b and 6c).

21 Previous studies using AFM^{6,7,15}, electron cryotomography^{16,17},
22 or scanning electron microscopy⁸ identified a variety of bacterial
23 surface features associated with initiation or completion of cell
24 division. To the best of our knowledge, inherited morphological
25 features associated with division site selection have not been
26 identified until now. Although the well-characterized Min and
27 Noc systems serve as negative regulators of FtsZ ring formation in
28 evolutionarily divergent bacteria, emerging evidence suggests that
29 these systems might not be responsible for initial specification of
30 the division site *per se*¹. Rather, these mechanisms may function
31 at later steps to help ensure that the FtsZ ring forms only at an
32 appropriate place (distant from the cell poles and membrane-tethered

DNA) and at the correct time relative to nucleoid segregation. What,
then, are the mechanisms responsible for specifying the future
division site? In *Streptococcus pneumoniae*, the MapZ protein
localizes as a circumferential band at midcell and sets the orientation
of the FtsZ ring¹⁸. However, most bacteria, including mycobacteria,
do not encode a MapZ homologue.

We show here that mycobacterial cell division occurs within
wave troughs on the undulating cell surface. Various bacterial
proteins are known to localize to negative¹⁹ or positive²⁰ membrane
curvatures. Mycobacterial proteins that target curved membranes
within wave troughs might serve as beacons for FtsZ ring assembly,
while proteins with preferential affinity for wave crests might serve
to repress division. Alternatively, peptidoglycan architecture might
direct the formation of surface undulations and mark wave troughs
as future division sites. In spirochetes, peptidoglycan crosslinking
has been reported to impact cell shape²¹ and to direct the division
machinery to an inherited zone of active peptidoglycan synthesis²².

Although mycobacterial wave troughs are preferred sites for cell
division, chromosomes also seem to play a negative regulatory role
in division site selection. Unlike wild-type cells, which always divide
within a centre-most wave trough, strains with defects in chromo-
some partitioning divide asymmetrically at an off-centre wave
trough when unpartitioned chromosomes are retained in the
distal cell half. These observations suggest that mycobacteria
might possess a mechanism analogous but not homologous to the
Noc system to prevent cell division over unsegregated chromo-
somes. Like Noc, this mechanism might serve as a ‘failsafe’ when
chromosome replication or partitioning is severely impaired²³, as
in ParB-deficient cells. In wild-type mycobacteria, nascent septa
have been observed to form over chromosomes that are still in the
process of segregating²⁴, and assembly of FtsZ rings over chromo-
somes has been observed in *E. coli* with diffuse nucleoids²⁵. These

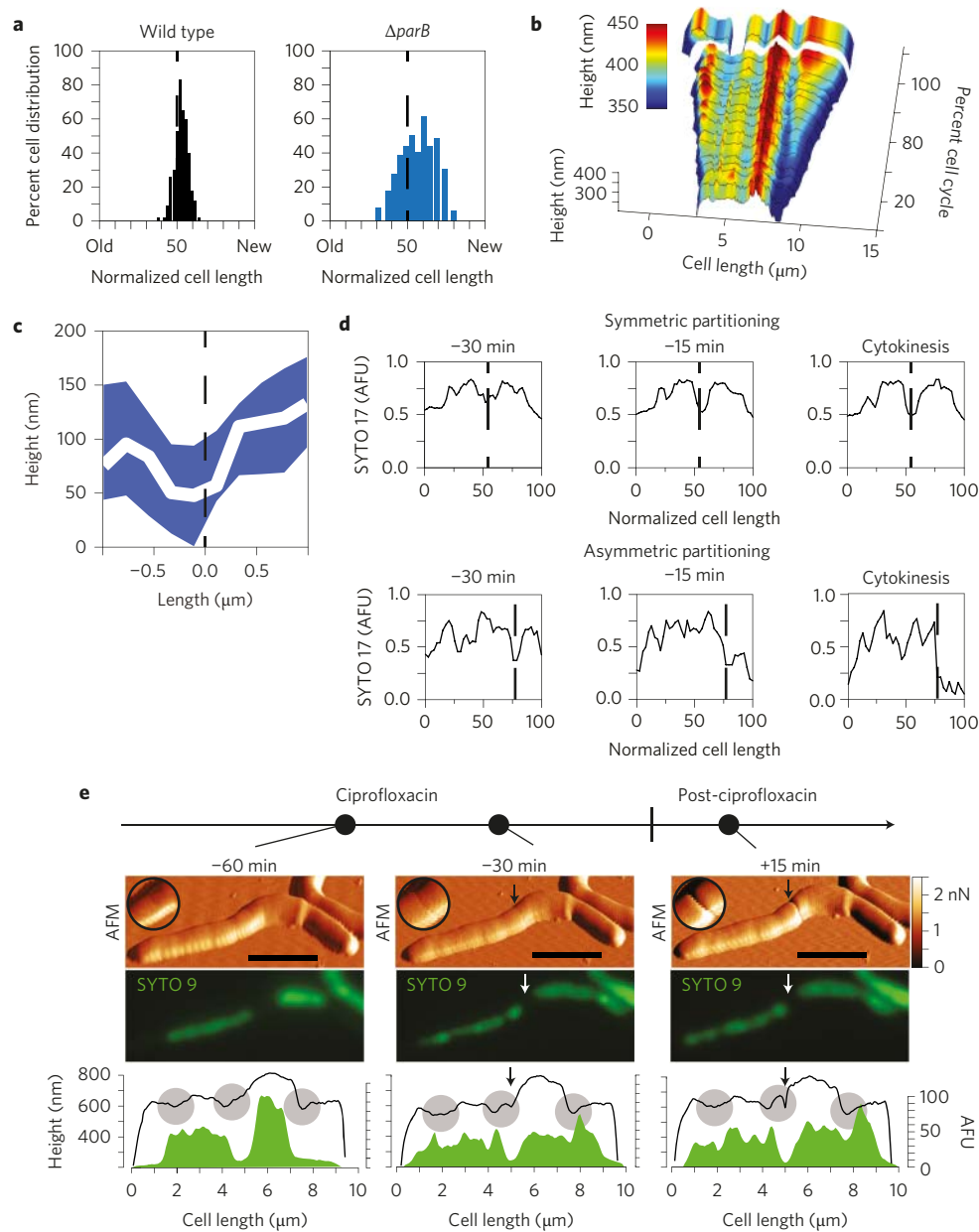


Figure 4 | Asymmetric divisions occur at off-centre wave troughs. **a**, Distribution of division site selection in wild-type cells (black histogram) ($n = 520$) and $\Delta parB$ cells (blue histogram) ($n = 278$). **b**, Kymograph of cell height of a representative $\Delta parB$ cell from birth to division, showing asymmetric division within an off-centre wave trough ($n = 27$). **c**, Averaged surface height around future sites of off-centre divisions ($n = 27$). White line: average height within a range $\pm 1 \mu\text{m}$ in each direction of the cleavage site. Blue background: variation (25th and 75th percentiles) in surface height. **d**, Representative graphs depicting the distribution of DNA along the cell length of $\Delta parB$ cells at 30, 15 and 0 min before cytokinesis. Top: examples of symmetric nucleoid partitioning ($n = 18$). Bottom: examples of asymmetric nucleoid partitioning leading to the formation of an anucleate new-pole daughter cell ($n = 48$). Dashed lines correspond to future division sites, which often correspond to local minima of chromosomal DNA. **e**, AFM (upper) and fluorescence (middle) images of a representative wild-type cell before (-60 and -30 min) and after ($+15$ min) release from a ciprofloxacin block. Longitudinal height profiles (black lines) stacked on top of DNA profiles (green plots) of the same cell show that the future division site (arrows) occurs at a local DNA minimum within a wave trough (grey shaded circle). AFM images are a three-dimensional representations of the height with the peak force error (PFE) signal overlaid as a skin. The PFE signal scale bar is expressed in nN. Scales bar (AFM images), $3 \mu\text{m}$. Image sequence is representative of $n = 7$.

1 observations suggest that the degree of nucleoid concentration or
 2 compaction might affect the activity of the nucleoid occlusion
 3 system. We propose a bipartite model of division site selection, in
 4 which cell-surface wave troughs are 'licensed' sites for cell division
 5 and segregated chromosomes suppress division at off-centre wave
 6 troughs. Although the molecular factors involved in division site
 7 selection in mycobacteria are unknown, the observation that cell
 8 divisions occur within wave troughs will refocus the search for
 9 such factors to a point much earlier in time than FtsZ ring

formation, which is currently the earliest known event in division
 site selection in rod-shaped bacteria.

Methods

Bacteria. *Mycobacterium smegmatis* mc²155 (wild-type) and derivative strains were grown in Middlebrook 7H9 liquid medium (Difco) supplemented with 0.5% albumin, 0.2% glucose, 0.085% NaCl, 0.5% glycerol and 0.05% Tween-80. Cultures were grown at 37°C to mid-exponential phase (optical density at 600 nm (OD_{600}) of ~ 0.5). Aliquots were stored in 15% glycerol at -80°C and thawed at room temperature before use. The $\Delta parB$ strain with an unmarked in-frame deletion of the

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1 *parB* gene has been described previously¹³. The *attB*-integrating plasmid
2 expressing a Wag31-GFP fusion protein has also been described previously².
3 The *Mycobacterium smegmatis* RipA conditional knockdown strain was cultured
4 and manipulated as described previously¹⁰. Wild-type cells were filamented
5 with 500 ng ml⁻¹ ciprofloxacin (Sigma) or 50 ng ml⁻¹ mitomycin C (Sigma).

6 **FtsZ-GFP reporter.** The open reading frame (ORF) encoding *M. smegmatis* FtsZ
7 was PCR-amplified using primers MsmftsZ-F (gctagcatgacccccgcataactacctcg) and
8 MsmftsZ-R (ggactgtctctctgacccctccaccgaaccaccaccctgaaccaccaccctgaaccaccggg
9 gtgccgcatgaaggcg) with genomic DNA as the template. The underlined sequence
10 in primer MsmftsZ-R represents the linker sequence encoding four repeats of
11 Gly-Gly-Ser-Gly-Gly. The amplicon was ligated into vector pCR2.1 (Invitrogen) and
12 verified by DNA sequencing, then excised as an *NheI*-*SpeI* fragment and ligated into
13 the unique *NheI* site in the *attB*-integrating vector pND250, which encodes a
14 hygromycin resistance marker. The resulting plasmid (pND275, provided by
15 N. Dhar) expresses *ftsZ-gfp* (in-frame fusion) from a strong anhydrotetracycline
16 (ATc)-inducible promoter. pND275 was electroporated into *M. smegmatis* and
17 transformants were selected by plating on solid medium containing 50 µg ml⁻¹
18 hygromycin (Sigma).

19 **Microscopy.** For time-lapse fluorescence microscopy, bacteria were grown to
20 mid-exponential phase (OD₆₀₀ ≈ 0.5) in 7H9 liquid medium, collected by
21 centrifugation (2,400g, 5 min), concentrated 30-fold in fresh 7H9 medium (37 °C)
22 and passed through a polyvinylidene difluoride syringe filter (Millipore, 5 µm pore
23 size) to remove clumps. The declumped bacteria were spread on a glass coverslip,
24 covered with a semipermeable membrane and cultured in a custom-made microfluidic
25 device with a continuous flow of 7H9 medium at 37 °C (flow rate, 25 µl min⁻¹),
26 as described previously²⁶. Nucleoid staining was accomplished by adding
27 SYTO 17 Red (170 nM final) in the flow medium. Bacteria were imaged at 15 min
28 intervals with a DeltaVision personal DV microscope (Applied Precision) equipped
29 with a ×100 oil-immersion objective and in an environmental chamber maintained
30 at 37 °C (ref. 2). Images were recorded on phase-contrast and fluorescence channels
31 (475/28 nm excitation and 525/48 nm emission filters for FITC; 575/25 nm
32 excitation and 632/22 nm emission filters for CY-5) with a CoolSnap HQ2 camera.

33 **AFM.** Coverslips were prepared by mixing polydimethylsiloxane (PDMS) (Sylgard
34 184, Dow Corning) at a ratio of 15:1 (elastomer:curing agent). Air bubbles in the
35 mixture were removed under negative pressure for 15 min. The PDMS mixture was
36 dropped onto a 22 mm glass coverslip (VWR) and spin-coated at 8,000 r.p.m. (SUSS
37 MicroTec LabSpin6)²⁷ for 30 s. PDMS-coated coverslips were baked at 80 °C for
38 10 min before use. A 3 ml aliquot of mid-exponential phase cell culture was filtered
39 through a 0.5 µm pore size PVDF filter (Millipore) to remove cell clumps and
40 concentrated into 200 µl final volume by pelleting cells (2,400g, 8 min). A 50 µl
41 aliquot was deposited on the hydrophobic surface of a PDMS-coated coverslip and
42 incubated for 20 min to increase the surface interactions between bacteria and
43 coverslip. A constant flow (140 µl min⁻¹) of 7H9 medium was supplied by a
44 syringe pump. Where indicated, isoniazid (Sigma) was added to the flow medium at
45 5 µg ml⁻¹ (1× MIC) or 50 µg ml⁻¹ (10× MIC). The flow medium was preheated in a
46 custom-made chimney that served as a bubble trap and heating element for
47 maintaining fluid at 37 °C in the sample space. Bacteria were imaged by a Peak Force
48 QNM with a Nanoscope 5 controller (Veeco Metrology) at a scan rate of 0.5 Hz and
49 a maximum Z-range of 5 µm. A ScanAsyst fluid cantilever (Bruker) was used.
50 Continuous scanning provided snapshots at 10 min intervals. Height, peak force
51 error, adhesion, dissipation, deformation, DMT modulus and log DMT modulus
52 were recorded for all scanned images. The peak force error yields a fine
53 representation of the height on the order of 10 nm in the Z axis; this is computed as
54 the difference between the peak force setpoint and the actual value. Images were
55 processed using a custom-made MATLAB program²⁸ or Gwyddion (Department
56 of Nanometrology, Czech Metrology Institute). ImageJ was used for extracting
57 bacterial cell profiles in a tabular form. MATLAB scripts were developed for
58 automating the analysis of experimental data sets and generating graphical
59 representations of data.

60 **Correlated fluorescence and AFM.** Correlated fluorescence and AFM images were
61 acquired as described in ref. 4. Briefly, fluorescence images were acquired with an
62 electron-multiplying charge-coupled device (EMCCD) iXon Ultra 897 camera
63 (Andor) mounted on an IX71 inverted optical microscope (Olympus) equipped
64 with an UAPON100XOTIRF ×100 oil immersion objective (Olympus) with the ×2
65 magnifier in place. Illumination was provided by an MLC (monolithic laser
66 combiner, Agilent) using the 488 or 561 nm laser output coupled to an optical fibre
67 with appropriate filter sets: F36-526 for GFP and F72-866 for FM4-64 (AHF
68 Analysetechnik). For membrane staining, 0.2 µg ml⁻¹ FM4-64 or 15 µg ml⁻¹ FM1-
69 43 (Life Technologies) was used. The AFM was mounted on top of the inverted
70 microscope, and images were acquired with a Dimension Icon scan head
71 (Bruker) using ScanAsyst fluid cantilevers (Bruker) with a nominal spring constant
72 of 0.7 N m⁻¹ in peak force tapping mode at a setpoint <2 nN and typical scan rates
73 of 0.5 Hz. The samples were maintained at 37 °C in 7H9 liquid medium heated by a
74 custom-made coverslip heating holder controlled by a TC2-80-150 temperature
75 controller (Bioscience Tools).

Cell measurements

Cell growth measurements. Cell length was measured as the sum of short linear
segments tracking along the midline of individual cells. Cell lengths at birth (L_b) and
division (L_d) were defined as distances between cell ends. Interdivision time (I_t)
was defined as the time between birth and division. Elongation velocity averaged over the
lifetime of the cell was defined as $(L_d - L_b)/I_t$. Elongation rate averaged over the
lifetime of the cell was defined as $(L_d/L_b)/I_t$. Elongation rate averaged over a specific
time interval was defined as $(L_n/L_i)/(t_n - t_i)$, where L_i is the initial cell length at time t_i
and L_n is the cell length at a later time t_n . Cell volume was calculated as the sum of the
cylindrical volume of each incremental pixel along the midline of the cell using the
height as the diameter. Volumes at birth (V_b) and division (V_d) were defined for each
cell. The velocity of volume change averaged over the lifetime of the cell was defined
as $(V_d - V_b)/I_t$. The rate of volume change averaged over the lifetime of the cell was
defined as $(V_d/V_b)/I_t$. The rate of volume change averaged over a specific time
interval was defined as $(V_n/V_i)/(t_n - t_i)$, where V_i is the initial cell length at time t_i ,
and V_n is the cell length at a later time t_n . Cell profiles were traced along the ridgeline,
defined as the highest point in the lateral axis following the length of the cell.

Identifying wave troughs. The longitudinal midline along the length of the cell was
extracted manually from the AFM height images for each individual cell at each time
point. To reduce the possibility of misidentifying small fluctuations between
adjacent height values as waveform undulations, we applied a moving average filter
with an averaging window of 100–200 nm. This smoothing treatment of the height
profiles did not affect the interpretation of our data, because the distances between
undulations are an order of magnitude greater than the smoothing window. The cell
profile was flattened by conducting a second-order polynomial fit of the height
profile (Supplementary Figs 9b and 18). The points falling below the curve fit and
exhibiting opposing slopes on either side are local minima. The second-order polyfit
localizes the wave trough position to less than 100 nm of the centre of a wave trough.
The points localized above the curve fit and exhibiting opposing slopes on either side
are local maxima. Wave troughs were annotated in at least two successive time points
within a certain spatial range corresponding to the relative increase in cell length
over the observed time period (see MATLAB code in file: Cell_Physiology_Analysis,
lines 175–179, within the Supplementary Section ‘Flatten the cell height’).

Identifying the central wave trough. The wave trough closest to the cell centre
throughout the life of the cell is defined as the central wave trough.

Calculating the average cell surface shape at the site of cell division. Average
dimensions of the central wave trough were calculated over the interdivision time
(birth to cell cleavage) by averaging the flattened surface height within a range of
1 µm to the left and right of the division position (see MATLAB code in file:
TroughProfile_Morphology). Data were collected at 197 distinct time points
throughout the interdivision times of all untreated wild-type cells. For ease of
graphical representation, data from time points were binned into 15 groups. Binned
data were graphed in a three-dimensional surface plot.

Plotting DNA distributions and identifying anucleate daughter cells. SYTO 17-stained
cells were imaged by fluorescence time-lapse microscopy and dual AFM-optical
microscopy. Cell profiles were obtained by tracing longitudinal lines along the
midlines of the cells. In $\Delta parB$ cells, highly asymmetric divisions may lead to the
formation of anucleate daughter cells, which were identified as cells with very low or
absent DNA signals that cease to grow and divide after birth.

Data availability. Raw experimental data are available at <https://figshare.com/s/e11e1063af5cd0d02295>. MATLAB scripts are accessible at <https://figshare.com/s/3d42ad95a892c641972b>.

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