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Cell division licensing in the multi-chromosomal *Vibrio cholerae* bacterium

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Cell division must be coordinated with chromosome replication and segregation to ensure the faithful transmission of genetic information during proliferation. In most bacteria, assembly of the division apparatus, the divisome, starts with the polymerisation of a tubulin homologue, FtsZ, into a ring-like structure at mid-cell, the Z-ring ¹. It typically occurs at half of the cell cycle when most of the replication and segregation cycle of the unique chromosome they generally harbour is achieved ². The chromosome itself participates to the regulation of cell division, at least in part because it serves as a scaffold to position FtsZ polymerisation antagonists ³. However, about 10% of bacteria have more than one chromosome ⁴, which raises questions about the way they licensed cell division ³. For instance, the genome of *Vibrio cholerae*, the agent of the cholera, is divided between a 3 Mbp replicon that originates from the chromosome of its mono-chromosomal ancestor, Chr1, and a 1 Mbp plasmid-derived replicon, Chr2 ⁵. Here we show that Chr2 harbours binding motifs for an inhibitor of Z-ring formation, which helps accurately position the *V. cholerae* divisome at mid-cell and postpones its assembly to the very end of the cell cycle.

The genome of *V. cholerae* encodes for homologues of all the cell division proteins of *Escherichia coli*, a mono-chromosomal bacterium from which much of our knowledge on bacterial cell division has been gained ². However, it was previously observed that a fluorescent protein fusion of *E. coli* FtsZ formed foci at the *V. cholerae* cell poles, in contradiction with its exclusive mid-cell polymerisation in *E. coli* ⁶. To confirm this observation, we inspected the localisation of Red

Fluorescent Protein (RFPT) and Yellow Fluorescent Protein (YFP) *V. cholerae* FtsZ C-terminal fusions. The fusions were produced from an ectopic locus in conjunction with the wild type *ftsZ* allele. They suppressed the lethality of a thermosensitive allele of *ftsZ* at the non-permissive temperature, demonstrating that they were at least partially functional (Figure S1a). Both fusions frequently located to one pole of the cells in *V. cholerae*, in contrast to the localisation pattern of FtsZ in *E. coli* (Figure 1a and Figure S2). The results were backed up by analysing the localisation of FtsK, a DNA pump that assembles as part of the divisome and pulls trapped chromosomes into the compartment where their origin of replication is located ^{7,8}. A Yellow-Green Fluorescent Protein (YGFP) *V. cholerae* FtsK C-terminal fusion was introduced in place of the wild type *ftsK*. It was fully functional (Figure S1b). It remained at the new pole for a longer time and relocated to mid-cell at a later stage than FtsZ-RFPT (Figure S2). Timing the progress of the cell cycle based on the cell length distribution of the observed population further suggested that FtsZ formed a Z-ring at mid-cell at a much later stage of the cell cycle in *V. cholerae* than in *E. coli* (Figure S2). Indeed, dual-colour time-lapse fluorescent microscopy demonstrated that FtsZ and FtsK remained located at the new cell pole, i.e. the pole resulting from the previous division event, for >10% of the cell cycle in *V. cholerae* (Figure 1b). In addition, a bright FtsZ signal only appeared at mid-cell at >80% of the cell cycle, concomitantly with the recruitment of the replication terminus of Chr1 at this position (Figure 1b). On the contrary, *E. coli* FtsZ formed a bright foci at mid-cell at 50% of the cell cycle (Figure 1b). Photo-activated localisation microscopy imaging indicated that *V. cholerae* FtsZ foci at new poles were not associated to a detectable

ring structure whereas bright mid-cell foci corresponded to Z-ring formation (Video S1). Together, these results suggested that the regulation of cell division in *V. cholerae* differs from that of previously studied mono-chromosomal species, such as *E. coli* ².

The genome of *V. cholerae* encodes for homologues of MinC, MinD and MinE, the major cell division regulators of *E. coli* ^{9,10}. In *E. coli* MinC inhibits FtsZ-polymerisation ^{10,11}. It forms membrane-associated complexes with MinD, a ParA-like ATPase. MinCD complexes oscillate from pole to pole, chasing *E. coli* FtsZ away from them ^{10,11}. The persistence of FtsZ and FtsK foci at the new pole of *V. cholerae* cells led us to suspect that the *V. cholerae* Min system might not be functional. Indeed, the localisation pattern of FtsZ and FtsK in *V. cholerae* was in some way similar to that of the cell division proteins in *Caulobacter crescentus*, which lacks a Min system ². In apparent agreement, *minCD* deletion barely altered the localisation pattern of FtsZ in *V. cholerae* (Figure 2a) and the stages of the cell cycle in which it left the new pole of the cell and formed a Z-ring at mid-cell (Figure 2b). In addition, it was associated with few cell division defects in *V. cholerae* whereas it led to massive mini-cell formation in *E. coli* (Table S1 and Figure 2c). Finally, the observation of an YGFP fluorescent fusion of *V. cholerae* MinD in conjunction with *V. cholerae* FtsZ-RFP suggested that MinCD complexes oscillated from pole to pole in *V. cholerae* (Video S2) but were unable to chase FtsZ away from them (Video S3). However, *minCD* deletion produced mini-cells when the arrangement of chromosomal DNA was perturbed by the inactivation of the HubP/ParAB1 partition system, which is responsible for the polar positioning of the replication origin region of Chr1 ^{12,13}, or

the inactivation of the MatP macrodomain system, which is responsible for the positioning of the replication terminus regions of Chr1 and Chr2 at the new pole of new born cells and their maintenance at mid-cell at the end of the cell cycle^{14,15} (Figure 2c and S3). Taken together, these results suggested that the *V. cholerae* Min system was active but that the arrangement of chromosomal DNA masked its role in cell division.

In many bacteria, a process referred to as nucleoid occlusion (NO) impedes cell division when the bulk of chromosomal DNA is still present at mid-cell¹⁶. The genome of *V. cholerae* encodes for a homologue of SlmA, the main NO effector of *E. coli*, which directly inhibits FtsZ polymerisation when bound to specific DNA motifs, the SlmA-Binding Sites or SBS^{10,17}. The deletion of *slmA* abolished the polar localisation of FtsZ in *V. cholerae* (Figure 2d and Figure S4a) and led to its earlier redirection towards mid-cell (Figure 2e and Figure S4a). In addition, the FtsZ fluorescent fusions concentrated into bright foci at mid-cell at an earlier time in $\Delta slmA$ cells than in wild-type cells, which suggested earlier Z-ring assembly (Figure 2e and Figure S4a). On the contrary, FtsZ left the new pole and assembled into a Z-ring at later stages of the cell cycle in cells in which a second copy of *slmA* was added at an ectopic locus (Figure S4a and S4b). Snapshot image analysis of *V. cholerae* cells ectopically producing a functional N-terminal YGFP SlmA fusion (Figure S5) showed that it always located over chromosomal DNA (Figure S6). However, it seemed excluded from cellular regions in which the terminus region of Chr1 was found (Figure 2f and S6). Correspondingly, the localisations of YGFP-SlmA and FtsZ-RFP were mutually exclusive (Figure 2g). Taken together, these observations suggested

that SlmA-mediated NO was a major regulator of cell division in *V. cholerae*.

We developed a whole-genome binding analysis method to determine the *V. cholerae* SBS motif and its distribution on Chr1 and Chr2 (Figure S7ab). In brief, sonicated *V. cholerae* genomic DNA was mixed with a purified N-terminal 6-histidine tagged version of *V. cholerae* SlmA that was shown to be functional (Figure S5). SlmA-bound and unbound DNA fractions were recovered and subjected to high-throughput sequencing. 79 peaks in the marker frequency profile of the SlmA-bound fraction corresponded to DNA fragments that were enriched by a factor of >3.5 compared to the unbound fraction (Table S2). Based on the assumption that their enrichment related to their affinity to *V. cholerae* SlmA, they were grouped in four categories. A motif similar (but not identical) to the *E. coli* SBS motif was found for each category at a median distance of 23 bp from the peak centres (Table S2). The motifs of the two more enriched categories were variants of the same 14 bp palindrome, 5'-AGTRAGCACTYAC-3', with the motif of the two less enriched categories corresponding to one half of the palindrome (Figure 3a). We verified *in vitro* that *V. cholerae* SlmA specifically bound to 5 of these sites, including one on Chr2, and that it had more affinity for a site highly enriched than for a poorly enriched site (Figure S7c and S8). All of these sites were better SlmA-binding substrates than a typical *E. coli* SBS (Figure S8). Based on these observations, we could draw a map of the distribution and strength of the *V. cholerae* SBS harboured by Chr1 and Chr2 (Figure 3b). They were concentrated on the origin proximal region of both chromosomes, with the notable exception of a strong SBS motif (SBS29) in the terminus region of Chr1. SBS29 deletion did not change FtsZ

localisation choreography (Figure S4c).

We previously determined the cellular arrangement and segregation choreography of Chr1 and Chr2^{12,14} (Figure 3c). Based on this knowledge, we calculated the location of each SBS along the long axis of the cell as a function of the cell length. We then modelled the cell cycle choreography of DNA-bound SlmA in *V. cholerae* using the enrichment of each SBS in the whole-genome SlmA-binding assay as its probability of occupancy by SlmA (Figure 3d). The model perfectly fitted with our cellular observations: in newborn cells, SlmA-bound DNA covered the whole length of the cell with the exception of the new pole, thus explaining FtsZ confinement to this location (Figure 2fg and 3d, Chr1+Chr2 panel, lower left white dashed region); SlmA-bound DNA was only excluded from mid-cell at a very late stage of the cell cycle, which explained the late Z-ring formation (Figure S6 and 3d, Chr1+Chr2 panel, middle right white dashed region). An advantage of our modelling strategy was to discriminate the respective contributions of Chr1 and Chr2 to SlmA-mediated NO. The sole presence of SBS on Chr1 could not account for the FtsZ cell cycle choreography: it left the whole younger half of the cell free from SlmA for a substantial part of the cell cycle and excluded SlmA from a large region around mid-cell at an early stage of the cell cycle (Figure 3d, Chr1 panel). However, Chr2 SBS were well positioned to confine FtsZ to the new pole of newborn cells and block Z-ring polymerisation until a very late stage of the cell cycle, suggesting that Chr2 SBS contributed to the regulation of cell division (Figure 3d, Chr2 panel).

To demonstrate the role of Chr2 SBS on the regulation of cell division, we checked whether cell division was altered in a synthetic mono-chromosomal

derivative (MCH1) of the wild type bi-chromosomal *V. cholerae* strain (N16961). MCH1 was obtained by the integration of most of Chr2 DNA in the replication terminus of Chr1, *ter1*; the dimer resolution site of Chr1, *dif1*, the origin of replication of Chr2, *oriC2*, and the partition genes of Chr2, *parAB2*, were deleted in the process ¹⁸ (Figure 4a). Previous work indicated that MCH1 incurred no increase in mortality and had a generation time only slightly longer than N16961 ¹⁸. Marker frequency analysis showed that replication of the MCH1 chromosome initiated at the replication origin of Chr1, *oriC1*, and progressed at a constant velocity to terminate near the dimer resolution site of Chr2, *dif2* (Figure S9a). Fluorescent microscopic observation of 11 loci along the fused chromosome (Figure S9bc) indicated that Chr1 DNA occupied almost the same territory in MCH1 as it did in N16961 at all stages of the cell cycle (Figure 4a). We modelled the choreography of DNA-bound SlmA molecules in MCH1 using the strategy we had adopted for wild type *V. cholerae*. The model suggested that SlmA molecules bound to Chr1 DNA occupied the same regions in MCH1 and wild type cells (Figure 4b, Chr1* panel). On the contrary, the subcellular arrangement of Chr2 DNA was completely altered, the whole of it adopting a segregation pattern similar to that of *ter1* in N16961 (Figure 4a and S9bc). As a result, SlmA molecules bound to Chr2 DNA were predicted to narrow the region where Z-ring assembly could take place at the time of division (Figure 4b, Chr2* panel) and to block access to mid-cell until after the segregation of the bulk of Chr2 DNA, at the very end of the cell cycle (Figure 4b, Chr2* panel). The model further revealed the presence of a SlmA-free zone starting at mid-cell in newborn MCH1 cells and ending at the $\frac{1}{4}$ and $\frac{3}{4}$ positions at the time of division

(Figure 4b, Chr1*+Chr2* panel, white dashed region). In agreement, the localisation of FtsZ was altered in MCH1 cells (Figure 4c). In particular, Z-rings often assembled before 50% of the cell cycle had elapsed, suggesting that normal cell division licensing was lost. In addition, important cell-to-cell variations were observed, which suggested an erratic positioning of FtsZ (Figure S10). Correspondingly, MCH1 cells displayed frequent asymmetric septum placement (Figure 4d). Indeed, about 1% of them showed two zones of constriction around mid-cell, which led to the formation of mini-cells (Video S4). Sister chromosomal DNA remained faithfully segregated in the majority of asymmetric cell division events. However, chromosomal DNA was removed from one of the two daughter cells - probably through the action of FtsK, during highly asymmetric division events (Video S5, bottom left cell). The deletion of *slmA* restored the accuracy of cell division in MCH1 cells, in agreement with the idea that their cell division defect was due to the perturbation of SlmA-mediated NO (Figure 4e, right panel, and S10c). On the contrary, the deletion of *minCD* aggravated the MCH1 cell division phenotype, suggesting that its action normally compensated for the altered localisation of Chr2 DNA in the strain (Figure 4e, left panel, and S10c). Note that septation is still biased to occur at mid-cell in $\Delta minCD$ MCH1 despite the presence of Chr2 DNA-bound SlmA at this position (Figure 4f). It suggests the existence of additional cell division regulatory systems. In support of this hypothesis, $\Delta minCD \Delta slmA$ *V. cholerae* cells are viable in both rich and poor medium (Table S1, Figure 2c and S5) in contrast to the synthetic lethality of *minCD* and *slmA* deletions in *E. coli*¹⁷. Chromosomal DNA by itself could play a negative role on Z-ring formation as proposed in *E. coli*^{17,19}.

Furthermore, the replication terminus of the chromosomes could play a positive role on Z-ring placement, as demonstrated in *E. coli* under conditions that suppressed synthetic lethality of a $\Delta minCD \Delta slmA$ mutant ²⁰.

The distinction that can be made between the origins of the different chromosomes of multi-chromosomal bacteria suggested the possibility that the replication and segregation cycle of a single chromosome, the one emanating from the unique chromosome of their mono-chromosomal ancestor, could orchestrate the licensing of cell division in these organisms. Faithful transmission of the genetic content of the other plasmid-derived chromosomes would then simply rely on the tuning of their replication and segregation cycles to that of the ancestral chromosome. However, initiation of the secondary chromosome of *V. cholerae* is delayed until after the concomitant replication and segregation of $\frac{2}{3}$ of its primary chromosome is achieved ²¹ (Figure 4f). Therefore, licensing of cell division at this stage, as observed in the mono-chromosomal bacteria so far studied, would jeopardise the faithful transmission of Chr2 sister chromatids. Our results suggest that this peril is avoided by the presence of SBS motifs on Chr2. Thus, it is the advancement of the replication and segregation cycle of Chr2 that dictates the timing of divisome assembly (Figure 4f). Investigation of cell division in several mono-chromosomal bacteria indicated that the mechanisms directing divisome assembly to the low DNA-density zone that develops between chromatid sisters are not conserved. In this regard, it will be interesting to know how cell division and chromosome segregation are coordinated in bacteria with divided genomes that arose from ancestors with fundamentally different cell division licensing

mechanisms.

Methods

Plasmids and strains

Bacterial strains and plasmids used in this study are listed in Tables S3 and S4, respectively. All strains are derivatives of the El Tor N16961 strain rendered competent by the insertion of *hapR* by specific transposition and constructed by natural transformation. Engineered strains were confirmed by PCR.

The FtsK-YGFP fusion was inserted in place of the endogenous *V. cholerae ftsK* allele. All the other fusion proteins were introduced at the *lacZ* or the *hapR* locus. HU α , LacI and ParBT1 fluorescent protein fusions were produced under the *E. coli lacZ* promoter, leakiness of the promoter was sufficient for imaging. FtsZ, SlmA and MinD fluorescent protein fusions were produced from the arabinose promoter using 0.02% of Arabinose.

Fluorescence microscopy

Cells were grown in M9 minimal medium supplemented with 0.2% fructose and 1 $\mu\text{g/ml}$ thiamine to exponential phase and spread on a 1% (w/v) agar pad (ultrapure agarose, Invitrogen) for analysis. For snapshot analyses, cells images were acquired using a DM6000-B (Leica) microscope and were analysed using MicrobeTracker²². At least two biological replicates were used for each experiment. For time-lapse analyses, the slides were incubated at 30°C and images acquired using an Evolve 512 EMCCD camera (Roper Scientific) attached to an Axio Observe spinning disk (Zeiss). At each time point, we took a stack of 32 bright-field images covering positions 1.6 μm below and above the focal plane. Cell contours were

detected and cell genealogies were retraced with a MatLab-based script developed in the lab ¹⁴. After the first division event, the new pole and old pole of cells could be unambiguously attributed based on the previous division events. To measure the percentage of mini-cell formation, cells were grown in LB medium. Pre-cleaned coverslip (#1.5H, Marienfeld) were used for PALM imaging. Acquisitions were performed with the N-STORM (Nikon) system and a SR Apo TIRF 100x (1.49 numerical aperture) oil objective. Images were taken using a TIRF inverted Nikon Eclipse Ti-E microscope coupled to an iXon DU897 EMCCD camera (Andor).

N-terminal 6-histidine tagged version of *V. cholerae* SlmA (6HisSlmA) purification

6HisSlmA was overproduced from plasmid pEYY20 in *E. coli* BL21DE3 Δ *slmA* cells grown at 30°C in LB medium. At mid-exponential growth gene expression was induced by the addition of 0.5 mM IPTG. After 2 hours of induction, cells were harvested, washed in 20 mM Tris-HCl pH 8, 1 mM PMSF, 1 mg/ml lysozyme, 0.05% Triton X-100 and EDTA-Free protease inhibitors (ROCHE) and lysed with a French press. The lysate was cleared by centrifugation for 30 minutes at 20000 rpm. The supernatant was loaded on a 5 ml HisTrap HP column (GE Healthcare), washed with 300 mM imidazole buffer and the protein eluted with a gradient of imidazole from 300 mM to 1 M. Fractions corresponding to pure 6HisSlmA were harvested and loaded on a 5 ml HiTrap Heparin HP column (GE Healthcare) and washed with 400 mM NaCl. The 6HisSlmA protein was eluted with a NaCl gradient from 400 mM to 1 M. Fractions corresponding to pure 6HisSlmA were dialysed in 20 mM Tris-HCl pH 8, 0.1 mM EDTA, 0.5 M NaCl, 20% glycerol, 1 mM DTT and stored at -80°C.

Whole genome *in vitro* binding assay

Genomic DNA (gDNA) was extracted from an overnight culture of *V. cholerae* N16961. 1 ml of a 0.5 mg/ml gDNA solution in a Covaris (micro or mini) tube was sheared in a Covaris focused-ultrasonicator with a duty cycle of 10%, a peak incident power of 140 W, a cycle burst of 200, a time of 1200 sec. Sheared DNA fragments were between 50 to 300 bp. The SlmA-binding reaction mix was prepared with the following components: 1 mg of 6HisSlmA protein (41.6 nmol), 1 mg of sheared gDNA (2 ml), 10X Binding buffer (20 mM Tris-Cl (pH 7.5), 1 mM EDTA, 150 mM NaCl, 0.1 mg/ml BSA)(0.4 ml), annealed competitor primers (5000 pmol; Table S5). The final volume was adjusted to 4 ml with water and the solution was incubated at 4°C for 1 h under rotation. The binding reaction mix was loaded on a 1 ml HisTrap HP column (GE Healthcare). The column was washed with 20 ml of a 10 mM phosphate buffer pH 8 and 250 mM NaCl buffer. Stepwise gradient elution was performed with NaCl concentrations from 250 mM to 1000 mM. 1 ml of each fraction was recovered and the DNA concentration quantified with a Qubit dsDNA HS Assay Kit (Invitrogen). The elution profile revealed the most specific fractions, which were combined. The NGS library was prepared and sequenced with a MiSeq (Illumina) by the IMAGIF Core facility.

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Author Contributions

Conceived and designed the experiments: EG, MP, YY, FXB. Performed the experiments: EG, MP, RLB, EP. Analyzed the data: EG, MP, YY, JMD, FXB. Contributed reagents/materials/analysis tools: EG, JMD, LM. Wrote the paper: EG, MP, YY, FXB.

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Figure Legends

Figure 1. Cell cycle choreography of *V. cholerae* FtsZ. **a.** Cellular localisation of FtsZ-RFPT in *V. cholerae* N16961 cells (left panel) and FtsZ-GFP in *E. coli* MG1655 cells (right panel) grown in minimal medium. Representative images of multiple biological and technical replicates. Scale bar = 2 μ m. **b.** Cell cycle choreography of FtsZ-RFPT (top left panel, compilation of 59 single cell cycles), FtsK-YGFP (top right panel, compilation of 59 single cell cycles), *ter1* locus (bottom left panel, compilation of 81 single cell cycles) in *V. cholerae* N16961 and FtsZ-GFP in *E. coli* MG1655 cells (bottom right panel, compilation of 49 single cell cycles) grown in minimal medium. In the heat maps, black corresponds to the lowest and dark red to the highest intensities. Y-axis: position along the cell length, with 0 corresponding to the new pole and 1 to the old pole. X-axis: cell cycle, with 0 corresponding to birth and 1 to scission.

Figure 2. FtsZ localisation in Δ *minCD* and Δ *slmA* *V. cholerae* cells. **a.** FtsZ-RFPT cellular localisation in *V. cholerae* N16961 Δ *minCD* cells grown in minimal medium. **b.** FtsZ cell cycle choreography in *V. cholerae* N16961 Δ *minCD* cells grown in minimal medium (compilation of 68 single cell cycles). **c.** Percentage of mini-cells formed at each division in rich medium as determined from 12 independent time-lapse experiments. Mini-cells examples are represented in Figure S3ab. Ec = *E. coli* MG1655, Vc = *V. cholerae* N16961. Error bars represent standard deviation of 3-5 experiments **d.** FtsZ-RFPT cellular localisation in *V. cholerae* N16961 Δ *slmA* cells grown in minimal medium. **e.** FtsZ cell cycle choreography in *V. cholerae* N16961 Δ *slmA* cells grown in minimal medium (compilation of 31 single cell cycles). **f.**

Minimal-medium cellular localisation of SlmA-YGFP and of a locus close to *dif1* (*ter1*) as a marker for the new pole in newborn cells (top panel) and dividing cells (bottom panel). **g.** Minimal-medium cellular localisation of SlmA-YGFP and FtsZ-RFPT. Scale bars = 2 μm . **a,d,f** and **g** are representative images of 2 biological and technical replicates.

Figure 3. *V. cholerae* SlmA Binding Sites genomic distribution and cell cycle choreography. **a.** Sequence logo of the consensus SlmA-binding motifs. Fragments that were enriched in the whole genome-binding assay were grouped in four categories on the basis that fold enrichment (*fe*) was related to *V. cholerae* SlmA affinity. **b.** Circular diagram of the *V. cholerae* chromosomes representing the SlmA binding sites. SlmA affinity for each site, as suggested by their fold enrichment, is indicated with circles of different sizes and colours. **c.** Schematic representation of the cellular arrangement of Chr1 and Chr2 in N16961 newborn and dividing cells grown in minimal medium. 1 corresponds to the old pole and 0 to the new pole. **d.** SlmA-bound DNA densities were modelled using the cell cycle choreography of single chromosomal loci, the location of each SBS and their affinity for SlmA. In the heat maps blue corresponds to low and dark red to high SlmA-bound DNA densities, white dashed lines delimit the SlmA-free regions where FtsZ localisation is permitted. Left panel: contribution of both Chr1 and Chr2 DNA; Central panel: sole contribution of Chr1 DNA; Right panel: sole contribution of Chr2 DNA. Schematic representations of Chr1 and Chr2 DNA arrangements are shown below the respective panels. Yellow: SBS-rich DNA; Green: SBS-poor DNA. White dashed lines delimit the DNA-bound SlmA free zones observed in the Chr1+Chr2 panel.

Figure 4. Altering the replication and segregation cycle of Chr2 perturbs the *V. cholerae* cell cycle. **a.** Schematic representation of the cellular arrangement of the single chromosome of MCH1 in newborn and dividing cells. 1 corresponds to the old pole and 0 to the new pole. **b.** SlmA-bound DNA cell cycle choreography in MCH1 is represented as described in Figure 3 for N16961. Chr1*: DNA originating from Chr1; Chr2*: DNA originating from Chr2. White dashed lines delimit the DNA-bound SlmA free zones of the Chr1*+Chr2* panel. **c.** Cell cycle distribution of FtsZ-YFP in MCH1 cells grown in minimal medium (compilation of 52 single cell cycles). **d.** Cell division accuracy histogram and representative phase-contrast image of MCH1 cells grown in minimal medium. Arrowheads mark constriction sites. Scale bar = 2 μ m. Percentages of cells with a constriction site distant from mid-cell by less than or more than 5% of the cell length are shown in black and red, respectively. Accuracy: total percentage of cells with a constriction site distant from mid-cell by less than 5%; n = number of analysed cells. **e.** Cell division accuracy histograms of Δ *minCD* (left panel) and Δ *slmA* (right panel) MCH1 cells grown in minimal medium. **f.** Schematic representation of the *V. cholerae* cell division and chromosome cycles. The Z-ring (represented as black dots) forms at mid-cell. Chr1 and Chr2 are replicated and segregated in opposite cell halves. Chr2 replication initiation is licensed after $\frac{2}{3}$ of Chr1 DNA has been replicated and segregated. Z-ring formation is licensed when the bulk of Chr2 has been segregated. Chr1 and Chr2 chromatid sisters are represented in dark and light blue and dark and light pink, respectively.







