Chromosome choreography during the non-binary cell cycle of a predatory bacterium

Graphical abstract

Highlights
- The *Bdellovibrio* chromosome is polarized, with ori located near the invasive pole
- The highly compacted nucleoid excludes cytosolic proteins in non-replicative cells
- Replication and segregation of chromosomes are uncoupled from cell division
- The centromeric protein ParB localizes at parS in a cell-cycle-dependent manner

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In brief
Kaljević et al. study non-binary proliferation in the predatory bacterium *Bdellovibrio bacteriovorus*. Examination of the spatiotemporal dynamics of its DNA throughout the cell cycle reveals that, as the predator elongates, concomitant rounds of replication and segregation generate chromosome copies for more than two daughter cells.
Chromosome choreography during the non-binary cell cycle of a predatory bacterium

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SUMMARY

In bacteria, the dynamics of chromosome replication and segregation are tightly coordinated with cell-cycle progression and largely rely on specific spatiotemporal arrangement of the chromosome. Whereas these key processes are mostly investigated in species that divide by binary fission, they remain mysterious in bacteria producing larger number of descendants. Here, we establish the predatory bacterium *Bdellovibrio bacteriovorus* as a model to investigate the non-binary processing of a circular chromosome. We found that its single chromosome is highly compacted in a polarized nucleoid that excludes freely diffusing proteins during the non-proliferative stage of the cell cycle. A binary-like cycle of DNA replication and asymmetric segregation is followed by multiple asynchronous rounds of replication and progressive ParAB-dependent partitioning, uncoupled from cell division. Finally, we provide the first evidence for an on-off behavior of the ParB protein, which localizes at the centromere in a cell-cycle-regulated manner. Altogether, our findings support a model of complex chromosome choreography leading to the generation of variable, odd, or even numbers of offspring and highlight the adaptation of conserved mechanisms to achieve non-binary reproduction.

INTRODUCTION

Bacteria thrive in highly diverse environments to which they finely adapt, as illustrated by the immense variety of proliferation modes that were selected through evolution. Despite this tremendous diversity, most of our knowledge about bacterial multiplication derives from work on a subset of model species, which all divide by binary fission: one mother cell elongates, duplicates its genetic information, and gives rise to two daughter cells upon a single cell division event. However, not all bacteria adhere to the simple paradigm of binary reproduction. Species from various lineages (including Actinobacteria, Cyanobacteria, and Bdellovibrionata) rely on sophisticated cell cycles involving multiple fission. Here, larger and sometimes variable numbers of progeny are generated from a polyploid mother cell, through several (sequential or synchronous) septation events. The non-binary proliferation of these species, which is inherently complex due to the production of more than two descendants, offers an attractive platform to shed light on overlooked cell-cycle regulation strategies in bacteria.

To achieve each cycle with precision, bacterial cells rely on specific and elaborate spatiotemporal organization. A prominent example of cellular organization in bacteria is the intricate coordination, in both space and time, of chromosome replication and segregation with other cell-cycle events, including growth and cell division. In model bacteria, the spatial and temporal organization of the chromosome depends on specific subcellular positions of key chromosomal loci, mainly the replication origin (*ori*) and terminus (*ter*), which display highly regulated dynamics during the cell cycle. Soon after replication initiation, most species studied so far (with the exception of γ-proteobacteria) employ the ParABS system to actively partition sister *ori*. In this system, directionality of *ori* segregation is provided by the exquisite interplay between the ParB protein, which binds and spreads from the centromeric *parS* sites near *ori*, and the unspecific DNA-binding ATPase ParA. Iterations of ParB-triggered ATPase activity and dissociation of ParA from the chromosome result in the segregation of duplicated *ParB-parS* complexes, before the replication forks reach the chromosomal *ter*. Specific mechanisms physically connect the partitioning of *ter* copies with cell constriction, thereby coordinating the last steps of chromosome segregation and cell division and ensuring that each daughter cell is equipped with a full set of genetic material. Except in some Streptotomycetes, the spatiotemporal organization of the chromosome and the interplay between fundamental cellular processes remain essentially unexplored in non-binary growing bacteria.
Figure 1. Linear arrangement of the B. bacteriovorus chromosome and localization of ori near the invasive pole
(A) Schematics of the Bdellovibrio bacteriovorus cell cycle. Numbers indicate key steps in the cycle: 1. Freely swimming attack phase (AP) cells. 2. Attachment of B. bacteriovorus to its prey, via pili located at the non-flagellated (invasive) pole. 3. B. bacteriovorus resides in the periplasm of the prey, which is now called bdelloplast. 4. Filamentous growth and consumption of prey content. 5. Pre-divisional state. 6. Non-binary division of the mother cell generates an odd or even number of daughter cells, which mature before 7. escaping the prey remnants and resuming the cell cycle. Attack phase (AP) and growth phase (GP) represent the G1 (non-replicative) and S (replicative) stages of the cell cycle, respectively. A G1-S transition takes place upon prey invasion (see Results).
(B) Schematics of the orthologous parS-ParB pairs used in this study to label chromosomal loci. The parSPMT1/parSP1 sequences (yellow/cyan) were integrated near the origin of replication (oriBb) or terminus of replication (terBb) and the cognate YFP-ParBPMT1 or CFP-ParBP1 fluorescent fusions were constitutively produced from a replicative plasmid, respectively. Strains carrying parSPMT1 or parSP1 are respectively referred to as ori::parSPMT1 or ter::parSP1. Loci where parSPMT1 or parSP1 were integrated are respectively referred to as ori or ter.
(C) ori and ter are polarly localized. Left to right: representative phase contrast and fluorescence images of AP cells of ori::parSPMT1 and ter::parSP1 strains expressing cognate YFP-ParBPMT1 and CFP-ParBP1 (GL868 and GL771, respectively); mean pole-to-pole profiles of relative fluorescence intensity of the corresponding fusion in the same cells; demographs of the corresponding fluorescent signal in the same cells sorted by length and oriented based on signal intensity. Heatmaps represent relative fluorescence intensities.
(D) ori and ter occupy opposite poles in most cells. Left to right: representative phase contrast and fluorescence images of AP cells of a ori::parSPMT1 ter::parSP1 strain expressing cognate CFP-ParBP1 and YFP-ParBPMT1 (GL995). Fraction of cells in which ori and ter colocalized is indicated (inset); fluorescence profiles as in (C); demographs as in (C), signals oriented based on YFP-ParBPMT1.
(legend continued on next page)
osmotic integrity within the bdelloplast, within the bdelloplast, B. bacteriovorus digests the prey content and grows as a filament before producing a variable, even or odd number of daughter cells, by multiple synchronous division events. Flagellated monoploid cells then exit the prey ghost and resume the cycle.

The filamentous growth and multiple progeny of B. bacteriovorus raise unexplored fundamental questions regarding the orchestration of chromosome-related processes during the cell cycle. What prevents AP cells from growing and replicating their DNA likely relies on a prey-triggered developmental switch that remains to be elucidated at the molecular level. After prey invasion, the single circular chromosome of each predator cell has to be copied and partitioned multiple times before releasing daughter cells, challenging the principle of temporal coupling between chromosome replication, segregation, and cell division. Remarkably, chromosome replication must generate odd or even numbers, in contrast with common exponential multiplication patterns. Whereas a few hints suggest that B. bacteriovorus exploits multiple replisomes at the same time during its growth phase, unambiguous insights into native replication dynamics are lacking. In addition, how and when chromosome segregation occurs relative to replication and cell division is unknown.

Here, we provide key insights into the spatial organization of the chromosome and the orchestration of chromosome replication and segregation during the intriguing life cycle of B. bacteriovorus. Using epifluorescence microscopy on living cells, followed by quantitative image analysis at the single-cell and population levels, we monitored the subcellular localization of ori and ter loci, as well as the native replication and segregation machineries, during the G1 (non-replicative), G1-S transition, and S (replicative) phases of the synchronized predatory cell cycle. Our results reveal the unusual polarity and compaction of its nucleoid, and shed light on a complex choreography of chromosome replication and ParABS-dependent segregation leading to the generation of a variable, even or odd number of offspring.

RESULTS

The chromosome of G1 predator cells features an unusual ori-ter polarity
to gain insight into the spatial organization of the chromosome in living B. bacteriovorus cells, we labeled the chromosomal origin (ori) and terminus (ter) using orthologous parS/ParB pairs. Here, the parSparMT1 or parSP1, sequence (from Yersinia pestis or the P1 prophage, respectively) was integrated in the chromosome of the wild-type HD100 strain near to the predicted ori or ter locus, respectively (see STAR Methods), and fluorescent fusions to the cognate parS-binding proteins ParBparMT1 and ParBpar1 were constitutively produced from a replicative plasmid. We first monitored the subcellular position of ori (YFP-ParBparMT1) or ter (CFP-ParBpar1) in living attack-phase (AP) cells, using epifluorescence microscopy. Each locus was detected as a single unipolar focus in most cells (Figures 1C and S1A), supporting the longstanding notion that AP cells carry only one copy of their chromosome and represent the non-proliferative (G1) phase of the cell cycle (Figure 1A). Similar analyses of fluorescence profiles in a series of control strains (carrying non-cognate parS/ParB pairs and/or lacking the parS tag) confirmed that foci correspond to specific parS/ParB pairs (Figures S1B and S1C), consistent with previous locus labeling data obtained with this system in other species.

These reporters also localized at the cell poles when the corresponding Parf fusion was produced at lower levels from a B. bacteriovorus AP-specific promoter (Figures S2A and S2B), excluding artifacts from overproduction. We found that ori and ter occupy opposite poles in the majority of the cells in which both loci were labeled (67% cells, n = 3,593 in a representative experiment) (Figure 1D). To determine the polarity of this ori-ter arrangement, we took advantage of RomR, an essential component of a predation complex at the invasive pole of B. bacteriovorus (Figure 2C). Interestingly, the ter marker localized at the pole opposite a RomR-tdTomato fusion in most cells (79% cells, n = 6,061 in a representative experiment) (Figure 1E). Consistently, images of G1 B. bacteriovorus cells attached to E. coli cells showed that ori occupies the invasive, non-flagellated pole, whereas ter localizes at the flagellated pole in the majority of cells (Figures 1F and S2D). Additional evidence for this orientation was obtained using the sheathed unipolar flagellum as a polarity beacon, labeled with membrane dyes in cells carrying the ori and/or ter labeling system (Figures 1G, S2E, and S2F). Taken together, these data show that in most G1 B. bacteriovorus cells, chromosomal ori and ter loci are positioned at the invasive and flagellated poles, respectively. Note that the preferential localization of ter is more flexible (at the flagellated pole in ~70% of cells regardless of the polar marker) compared to the strict localization of ori at the

(E) ter localizes at the pole opposite RomR in most cells. Left to right: representative phase contrast and fluorescence images of AP cells of ter:parSP1, strain expressing RomR-TdTomato and CFP-ParBpar1 (GL816). Fraction of cells in which RomR and ParBpar1 colocalized is indicated (inset); fluorescence profiles as in (C); demographs as in (C), signal oriented based on RomR-TdTomato.

(F) ori occupies the invasive pole and ter occupies the flagellated pole during prey attachment. Representative phase contrast and fluorescence images of AP cells of ori:parSP1ter:parSP1 strain expressing cognate CFP-ParBpar1 and YFP-ParBparMT1 (GL995) 30 min after mixing with prey.

(G) ori occupies the non-flagellated pole and ter occupies the flagellated pole. Representative phase contrast and fluorescence images of AP cells of ori:parSP1ter:parSP1 strain expressing cognate CFP-ParBpar1 and YFP-ParBparMT1 (GL995) after staining with FM4-64. Arrowheads point at ori and ter foci at opposite poles.

(H) Histogram of the relative distance from fluorescent spot of RomR-TdTomato (red), CFP-ParBpar1 (cyan), and YFP-ParBparMT1 (yellow) to the nearest cell pole for cells in (C) and (E); mean and SD values are shown. For all panels, schematics illustrate the relevant ori and ter labeling construct. Scale bars, 1 μm. n, number of cells analyzed in a representative experiment. For all experiments were performed at least twice. For all panels, cell outlines were obtained with Outli. See also Figures S1 and S2.
invasive pole. Strikingly, _B. bacteriovorus_ features an inverse chromosomal polarity compared to other species carrying one (or more) unipolar flagellum, in which the chromosomal centro- mere is always located at the flagellated pole of newborn cells (e.g., _Caulobacter crescentus_,_,11_47 _Vibrio cholerae_,_,48_ and _Agrobacterium tumefaciens_).55

The chromosome of _B. bacteriovorus_ is packed in a dense nucleoid that partially excludes freely diffusing proteins

Demographics and fluorescence profiles representing the subcellular localization of _ori_ and _ter_ loci (Figure 1C) or the polar marker RomR (Figure 1E) suggest that _ori_ and _ter_ occupy slightly off-pole positions. Indeed, these loci were more distant from the closest cell pole than RomR (Figure 1H). This is consistent with the idea that the chromosome of _B. bacteriovorus_ forms a nucleoid that does not fill the entire cytoplasm, as previously proposed.49,50 Yet, this aspect of the _B. bacteriovorus_ chromosome was never explored in living cells. Staining the DNA of G1 cells with fluorescent dyes confirmed the existence of a well-defined nucleoid occupying a fraction of the cytoplasm (Figures 2A, 2B, and S3A), with _ori_ and _ter_ foci marking the tips of the nucleoid (Figures 2C, S3B, and S3C).

Nucleoid area in G1 _B. bacteriovorus_ cells is smaller than measurements reported in other species so far,51 including _E. coli_ (Figures 2A and 2D). Genome sizes of _B. bacteriovorus_ and _E. coli_ are roughly similar (3.8 Mb and 4.6 Mb, respectively), indicating that the chromosome meshwork is more compact in _B. bacteriovorus_.

Remarkably, we found that freely diffusing fluorescent proteins were at least partially excluded from the _B. bacteriovorus_ nucleoid (Figure 2E), regardless of the fluorescent protein or the DNA dye (Figures S3D and S3E). We hypothesize that this intriguing distribution might result from the highly condensed DNA network. Although it is well established that the mobility of large molecular complexes such as ribosomes,52–54 protein aggregates,51,55,56 or higher order assemblies57,58 is impeded by nucleoids, this is the first report, to the best of our knowledge, of relatively small monomeric proteins being excluded.

Spatial arrangement of the chromosome is maintained during the G1–S transition and DNA replication initiates at the invasive pole

We subsequently set out to investigate chromosome dynamics further in the cell cycle when the predator cell resides within its prey, where it is expected to replicate its genomic content. To directly track DNA replication in living cells, we first monitored the subcellular distribution of DnaN, the replisome β-clamp commonly used as a proxy for replisome assembly and dynamics.59 We designed a scarless _dnaN::dnaN-msfGfp_ construct at the native chromosomal locus in a wild-type background, allowing the production of a DnaN-msfGFP fusion in place of the endogenous DnaN. DnaN-msfGFP signal was diffuse in the cytoplasm in G1 cells (exhibiting the partial nucleoid exclusion described above) (Figure 3A), indicative of unassembled replisome and consistent with the absence of DNA replication in G1. A DnaN-msfGFP focus appeared at one cell pole 95 ±
14 min after mixing with an *E. coli* prey cell (from 3 independent time-lapse experiments, total n = 318 bdelloplasts) (Figure 3B), marking replisome assembly and DNA replication initiation. We define the period between prey entry and initiation of DNA replication as the G1-S transition. During that period, the chromosome of *B. bacteriovorus* was still compact, and ori-ter polarity was maintained (Figure 3C). Importantly, we obtained several lines of evidence providing unambiguous support to the previously proposed idea that DNA replication initiates at the invasive pole40: (1) fluorescent foci of DnaN and RomR fusions occupied the same pole (98% cells, n = 133) (Figure 3D), (2) the DnaN-msfGFP focus colocalized with ori (Figure 3D), which was labeled either with parSPMT1-YFP-ParBPMT1 (colocalization in 99% cells, n = 104) (Figure 3D) or with the centromeric protein ParBbb (see below; colocalization in 93% cells, n = 111) (Figure S5D), and (3) Click-labeling of the thymidine analog 5-ethyl-2′-deoxyuridine (EdU) revealed a DNA synthesis spot that colocalized with the ori region (Figures 3E, S3G, and S3H). These results also validate the use of the parSPMT1-ParBPMT1 pair as proxy for the chromosomal ori in *B. bacteriovorus*. Thus, the spatial arrangement of the chromosome seen in G1 cells is preserved during the G1-S transition upon prey invasion.

Replisome dynamics reveal sequential firing of replication rounds and co-existence of multiple active replisomes in the growing predator

Examination of hundreds of predator cells imaged in time-lapse upon DNA replication initiation revealed common subcellular...
patterns. First, the DnaN-msfGFP focus migrated from the invasive pole to a midcell position (Figure 4A, arrowhead), likely reflecting the progression of replication along the mother chromosome.60,61 A second DnaN-msfGFP focus was detected on average 167 ± 15 min post-mixing with prey (i.e., 71 ± 5 min after the first focus), usually at the invasive pole (in 73.9% of 318 bdelloplasts from 3 independent time-lapse experiments) (Figure 4A, arrow for one representative bdelloplast). Transient splitting or merging of foci was occasionally observed, possibly representing the two replication forks, as reported in Caulobacter crescentus.62 A third DnaN-msfGFP spot formed at the opposite pole (Figure 4A, asterisk), followed by additional DnaN-msfGFP foci, which showed highly dynamic movements, indicating that more than two replisomes are simultaneously active in the growing predator cell (Video S1). The replication initiation steps do not seem to follow a readily predictable spatiotemporal pattern beyond the first two rounds, and new DnaN foci usually appeared sequentially instead of simultaneously, suggesting asynchronous firing of replication initiation (Figure 4A; Video S1). Similar dynamics were obtained when a fluorescent fusion of the clamp-loader component DnaX was used to label the replisome, as done in other species,63–66 although the signal intensity of DnaX-msfGFP (replacing the native DnaX, dnaX::dnaX-msfGFP) was weaker than the DnaN fusion (Figure S4A). The gyrase inhibitor novobiocin, which specifically blocks DNA replication initiation,67 prevented the formation of the first or subsequent DnaN-msfGFP foci depending on when the drug was added in the course of the cell cycle (Figure S4B). Thus, the growing number of replisomes during the S-phase shows that multiple rounds of DNA replication can occur concomitantly, and we hypothesize that these initiated asynchronously.

Figure 4. Multiple concomitant rounds of DNA replication in B. bacteriovorus
(A) Replisome dynamics during the proliferative (S) phase. B. bacteriovorus strain dnaN::dnaN-msfGFP (GL673) was mixed with prey and imaged in time-lapse after 43 min at 5-min intervals. Top: phase contrast and fluorescence images of selected time points of a representative experiment are shown. Arrowhead points to a mid-cell positioned DnaN-msfGFP focus; arrow points to the second DnaN-msfGFP focus; asterisk points to the third DnaN-msfGFP focus. The full time-lapse is shown in Video S1. Bottom: number of DnaN-msfGFP spots detected in Outfi over time (left) and kymograph of the DnaN-msfGFP signal along the cell length (right), for the same cell. Scale bar, 2 μm. See also Figure S6F.
(B and C) Loss of nucleoid exclusion suggesting chromosome decondensation during the proliferative (S) phase.
(B) Time-course experiment with strain Bd0063-0064::pBioFab-mcherry (GL1025). Cells were mixed with prey and stained with DAPI every 30 min, prior imaging. Top: phase contrast and fluorescence images of selected time points from a representative experiment are shown; white asterisks point to nucleoid exclusion of free mCherry at a time point prior DNA replication initiation; magenta asterisks point to clear nucleoid separation before cell division. Bottom: fluorescence intensity profiles of the corresponding signals in the same cells. Exclusion of mCherry from the DAPI signal is visible at 60 min but is less evident at 120- and 150-min. Scale bar, 2 μm. For all, outlines of B. bacteriovorus and bdelloplasts were drawn manually based on phase contrast images.
(C) Average pole-to-pole profiles of mean relative fluorescence intensity of mCherry for the indicated number of cells (n) at representative time points from the experiment described in (B). Exclusion of mCherry is visible at 0–30 min, but is not visible at 90 and 120 min when replication is ongoing. See also Figure S4 and Video S1.
Nucleoid decompaction may occur after DNA replication initiation

Dynamic replication factories (Figure 4A) need access to the chromosome, raising the question of whether nucleoid compaction might change upon G1/S transition. To gain insight into nucleoid dynamics during growth inside the prey, we imaged DAPI-stained cells of a strain constitutively producing free mCherry (to label the whole predator cell), during a time-course of prey infection. Early after DNA replication initiation, the nucleoid still occupied a restricted area in the cell from which the mCherry signal was partially excluded (Figures 4B, 4C, and S4C). At later stages (when multiple replisomes had been observed) (Figure S4C), the mCherry and DAPI signals overlapped without obvious exclusion pattern, suggesting an expansion of the chromosome meshwork compatible with mCherry diffusion (Figures 4B and 4C). Of note, areas that were the least stained with DAPI, presumably corresponding to regions of lower DNA density, were often occupied by a DnaN-msGFP-labeled replisome (Figure S4C, arrowheads). Nucleoid segregation and, conceivably, re-compaction of the chromosomes (marked by distinct DAPI-stained units) was visible at late time point’s prior division (Figures 4B, asterisks, and S4C, arrowheads). Based on these observations, we propose that nucleoid de-condensation might occur in growing Bdellovibrio cells, possibly triggered by the progression of replisomes and followed by re-compaction of the chromosomes before cell division.

Duplicated ori undergo asymmetric polar segregation and ter segregation is uncoupled from cell division

The simultaneous occurrence of multiple replication events in the growing predator cell raises the question of how and when the newly synthesized chromosomes are segregated. Because the subcellular positions of ori and ter loci determine the dynamics of chromosome segregation in other species,2−7 we examined the spatial arrangement of the chromosome during the proliferative (S) phase of the cell cycle. Time-lapse imaging of labeled ori showed that a second focus appeared and quickly moved toward the opposite pole (Figures 5A and S5A; Video S2), reminiscent of the asymmetric ori segregation described in several binary-dividing species.11,15,17,18,68,69 In line with this model,24,47 the ter focus shifted from its polar position to midcell (Figures 5B and S5B). Interestingly, this first round of ter segregation was achieved to completion, as the ter copies clearly split in two distinct foci at midcell (Figures 5B; Video S3). Thus, ter segregation is temporally uncoupled from cell constriction.

To further investigate segregation dynamics, we turned to the endogenous ParB (here named ParBbb) to mark the native centromeric parSbb site located next to ori (Figure S1A). Consistent with the reported biphasic expression pattern of the corresponding operon,70,71 we could only detect weak fluorescent signal in G1 cells of a strain in which parBbb was replaced by parBbb-mCherry (Figure S5C). Nevertheless, although no detectable foci could be observed in the G1 phase (see below), ParBbb-mCherry always formed a focus that colocalized with the first DnaN-msGFP-labeled replisome (Figure S5D). This is consistent with the colocalization of DnaN and ori shown previously. Furthermore, the first duplication and polar segregation of ParBbb-mCherry foci showed similar dynamics as parSpmT1-YFP-ParBpmT1-labeled ori (Figures 5C, 5D, and S6E) supporting our previous finding of asymmetric ori segregation and the use of parSpmT1 YFP-ParBpmT1 and ParBbb-mCherry as reliable ori reporters. Monitoring the dynamics of both ParBbb and ter in the same cells showed that ter relocation to midcell started after the newly duplicated ori reached the non-invasive pole (Figure 5E, time point, 200 min).

Chromosome segregation occurs progressively as new copies are being synthesized

After the first “binary-like” replication and segregation round, additional ParBbb foci gradually appeared (Figures 5C and 5D). The highest number of ParBbb foci varied between bdelloplasts and reached 5 on average under these conditions (n = 69, representative time-lapse experiment) (Figure 5C). As expected, the ParBbb-mCherry foci always colocalized with YFP-ParBpmT1-labeled ori on snapshots taken at various times during the proliferative phase of the cell cycle (Figure S5E). Strikingly, ParBbb foci were always evenly distributed during filamentous growth (Figure 5C; Video S4), indicating that even after the first round of replication, ori segregation occurs as soon as new chromosomal copies are being synthesized. In addition, temporal uncoupling of ter segregation from cell division was not limited to the first replication round, as additional ter foci appeared before visible cell constriction (Figure S5B, asterisks). We did not observe ter foci at the poles of the growing cell, unlike ori foci (Figures 5C and S5B, asterisk), hinting that ori but not ter will occupy the two “old” poles transmitted from the mother cell to the progeny. In the last minutes before non-binary cell division, ter-associated signal dispersed, and foci reappeared in the progeny (Figure S5B), which could suggest a temporary reorganization of the ter macrodomain during the division process.

Perturbations in the ParABS system impact progressive ori segregation and accurate cell-cycle progression

The asymmetric pole-to-pole segregation of the first duplicated centromere and the progressive partitioning of additional copies strongly suggest that the ParABS system drives these segregation events in B. bacteriovorus. To examine this idea further, we introduced perturbations in that system by constitutively producing ParBbb fusions from a plasmid that is expected to modify the ParA:ParB interplay.72,73 Overproduced ParBbb-FP fusions formed distinct foci in predator cells after prey invasion (Figure 6A, arrowhead), which colocalized with the YFP-ParBpmT1-labeled ori copies (Figure S6A). However, the localization pattern of these ParBbb-ori complexes (Figure 6A, asterisks) differed from cells in which ParBbb is produced at native levels, consistent with segregation defects: (1) in longer cells the second ParBbb focus rarely reached the opposite pole and instead stalled in the middle of the cell (Figures 6A and 6B), and (2) the number of foci did not regularly increase before division, and fusions of existing foci were observed (Figure 6A, time point 290 min). Moreover, constitutive production of ParBbb, either untagged or in fusion with mCherry or msGFP, led to pronounced phenotypes in the released progeny: (1) they displayed more variable and on average larger cell length and nucleoid area than control strains (Figures 6C, S6B, and S6C), and (2) cells had aberrant numbers of ori foci (Figure S6D). Altogether, our
data are consistent with the idea that the ParABS system is required to achieve multiple progressive rounds of asymmetric ori segregation in *B. bacteriovorus*. Of note, nucleoid exclusion of cytoplasmic proteins was still observed in ParB*Bb*-overexpressing cells, hinting that the mechanism of chromosome compaction is independent of ParAB*-mediated chromosome segregation (Figure S6E).

**Figure 5. Spatio-temporal arrangement of the chromosome during the proliferative phase of the cell cycle in *B. bacteriovorus***

(A) First round of ori segregation is asymmetric. *B. bacteriovorus* strain ori::parSP1, expressing cognate YFP-ParBP1 (GL868) was mixed with prey and imaged in time-lapse after 60 min with 10-min intervals. Phase contrast and fluorescence images of selected time points are shown; arrowheads point to the ori focus before replication and the duplicated ori foci after segregation; asterisk points to the ori copy being segregated toward the opposite pole. The time-lapse is shown in Video S2.

(B) ter segregation is temporally uncoupled from cell division. *B. bacteriovorus* strain ter::parSP1, expressing cognate CFP-ParBP1 (GL771) was mixed with prey and imaged in time-lapse after 60 min with 8-min intervals. Phase contrast and fluorescence images of selected time points are shown; arrowhead and asterisk point to mid-cell ter localization and segregation, respectively. The time-lapse is shown in Video S3.

(C) ParB-driven ori segregation during S phase. *B. bacteriovorus* strain parBBb::parBBb-mCherry (GL906) was mixed with prey and imaged in time-lapse after 60 min with 8-min intervals. Left: phase contrast and fluorescence images of selected time points are shown. Arrowheads point to the first ParBBb-mCherry focus, two segregated ParB foci, then 3 segregated foci; multiple well-separated ParBBb-mCherry foci are visible at time-point 444 min. The time-lapse is shown in Video S4. Right: number of ParBBb-mCherry spots detected in Oufi over time, for the same representative bdelloplast; average number of ParBBb-mCherry spots detected in Oufi, over time; gray area indicates SD; n indicates the average number of bdelloplasts analyzed until time point 384 min, from which the number of bdelloplasts that could be analyzed progressively decreased to 62.

(D) Kymograph of the ParBBb-mCherry signal along the cell length for one representative cell. Arrowheads indicate timing of, from left to right, the first ParBBb-mCherry focus, pole-to-pole segregation upon duplication, and the third focus.

(E) ter relocation to mid-cell starts after the second ori reaches the non-invasive pole. *B. bacteriovorus* strain parBBb::parBBb-mCherry ter::parSP1, expressing cognate CFP-ParBP1 (GL368) was mixed with prey and imaged in time-course with 30-min intervals. Phase contrast and fluorescence images of selected time points are shown; pink arrowheads point to ori copies; blue arrowheads point to ter copies. Schematics illustrate the ori and ter labeling constructs used in each panel. Scale bars, 1 μm. For all, outlines of *B. bacteriovorus* and bdelloplasts were drawn manually based on phase contrast images. See also Figure S5 and Videos S2, S3, and S4.
protein amounts. This finding raises the intriguing hypothesis that endogenous ParB<sub>Bb</sub> is unable to accumulate at parSB<sub>Bb</sub> sites during the G1 phase of the cell cycle. To get spatiotemporal insight into ParB<sub>Bb</sub> focus formation relative to cell-cycle progression, we constructed a strain producing both DnaN-msfGFP and ParB<sub>Bb</sub>-mCherry as single copies from their native chromosomal locus. ParB<sub>Bb</sub> does not localize at parSB<sub>Bb</sub> sites before the onset of DNA replication in <i>B. bacteriovorus</i> because the ParB<sub>Bb</sub>-mCherry signal formed a first detectable focus after DnaN-msfGFP (41 min on average, from single-cell analysis in double-labeled strains, Figure 7A; or when comparing population averages of single-labeled strains, Figures S6F and S6G; note some cell-to-cell variability, Figures S6F and S6G). However, ParB<sub>Bb</sub> does not require an active replisome to sustain accumulation at parSB<sub>Bb</sub>, because we could still detect ParB<sub>Bb</sub>-mCherry foci at the end of the S phase when the DnaN-msfGFP foci disassembled (Figure 7A). The ParB<sub>Bb</sub>-mCherry signal became diffuse after cell constriction started (Figures 5C and 7A), consistent with the absence of focus in G1 cells. Thus, our data show that in <i>B. bacteriovorus</i>, ParB<sub>Bb</sub> does not accumulate on its cognate parSB<sub>Bb</sub> sites during the G1 phase and G1/S transition.

**Multiple ori copies serve as platforms for replication rounds**

Finally, we asked how multiple chromosome replication events were orchestrated over time in growing <i>B. bacteriovorus</i> cells (i.e., whether DNA replication initiation steps were biased toward a specific subset of ori copies). Observation of bdelloplasts imaged in time-lapse showed that a DnaN-msfGFP spot colocalized with a ParB<sub>Bb</sub>-mCherry-labeled ori at several places in the cell (n = 65 in a representative experiment) (Figure 7A, asterisks), suggesting that distinct ori loci can serve as replication initiation platforms. Although several DnaN-msfGFP foci often clustered near the cell ends (Figure 7A, see Discussion), distinct foci were observed in other cell regions. Consistently, the ori copies marked by both ParB<sub>Bb</sub>-mCherry and DnaN-msfGFP (probably representing ori being replicated) occupy diverse subcellular positions and vary in number over time and among bdelloplasts (Figure 7A). Altogether, our data prompt us to propose that the asynchronous initiation of DNA replication from different ori platforms results in a non-exponential increase of chromosome numbers, consistent with odd or even numbers of daughter cells being released at each generation (Figure 7B).

**DISCUSSION**

In this study, we benchmarked the use of key fluorescent reporters to monitor the subcellular dynamics of chromosomal loci as well as the replication and segregation machineries in living <i>B. bacteriovorus</i> cells. Semi-automated analysis of intracellular features at the single-cell and population levels allowed us to shed light on how the chromosome is organized in space and time during the cell cycle, opening the way for future quantitative cell biological approaches in this bacterium. Taken together, our data suggest a model (Figure 7B) in which asynchronous initiation of multiple DNA replication rounds is sufficient to elucidate why
Figure 7. Stochastic chromosome replication initiation at multiple ori and progressive segregation producing odd or even offspring

(A) ParBBb forms foci after DNA replication initiation and multiple copies of ori can serve as a template for the next replication round. B. bacteriovorus strain dnaN::msfGFP parBBb::mCherry (GL1055) was mixed with prey and imaged in time-lapse after 75 min with 8-min intervals. Top: left: phase contrast and fluorescence images of selected time points are shown. Arrowheads point to fluorescent foci; asterisks and two-colored arrowheads point to colocalization of the corresponding fluorescence signals; right: number of DnaN::msfGFP (yellow) and ParBBb::mCherry (pink) spots detected in Oufit, over time for the same representative bdelloplast; SuperPlot representation of the time of appearance of first DnaN::msfGFP and ParBBb::mCherry foci and the time difference between them, in single cells of the GL1055 strain; the average for each signal is represented as a colored diamond; n indicates the number of cells analyzed in this experiment. Bottom: mean pole-to-pole profiles of relative fluorescence intensity of the corresponding fusions in the same cells; asterisks point to colocalization of the corresponding fluorescence signals. Scale bar, 1 μm. Outlines of B. bacteriovorus and bdelloplasts were drawn manually for display based on phase contrast images.

(B) A model for non-binary chromosome choreography in B. bacteriovorus. The highly condensed nucleoid of G1 (AP) cells is arranged such that ori (yellow) occupies the invasive pole and more flexible ter (blue) occupies the flagellated pole. Once inside the prey, cells experience a G1-S transition during which the state of the chromosome is apparently unchanged. At the beginning of the proliferative S (GP) phase, DNA replication starts from the invasive pole and the duplicated ori is segregated asymmetrically. Nucleoid visibly decondenses after DNA replication initiation. When the 2nd ori reaches the opposite pole, the replisome is at mid-cell. The 1st ter then moves from pole to mid-cell where it colocalizes with the 3rd ori copy, which was newly synthesized and segregated (usually from the same invasive pole). Progressive ori and ter segregations continue, following new DNA replication rounds where variable numbers and copies of ori serve as initiation platform, leading to odd or even ploidy. Distinct nucleoids are visible again before division of the mother cell by multiple fission. Nucleoid schematic for last two cells in S phase is omitted for clarity.
See also Figure S6.
suggests that the “mother” ori present at this pole is somehow primed, favoring re-initiation of chromosome replication at this location compared to the newly synthesized ori. Consistent with this idea, additional replisomes often accumulated from that pole in the growing predator, and later from the opposite pole, although DnaN foci were observed in other regions of the cell as well. What governs such spatial organization of DNA replication remains to be discovered. Considering the importance of transmitting a single and complete chromosome to each daughter cell, the dynamics of replication initiation are likely not determined by chance. We think that complex regulation occurs in both space and time to prevent replication from all oris at the same time, and most importantly to avoid starting new synthesis that would prematurely end when prey resources are exhausted. Even though the temporal control of the S phase with respect to cell-cycle progression and synchronous divisions is still unclear, our data provide clues into this question as we show that the late steps of chromosome segregation and cell division are uncoupled. In line with this idea, the positioning of one ori at both old poles of the pre-divisional mother cell inevitably results in at least one septum not being placed between two ter copies (Figure 7B).

Our study also revealed insights into the spatial organization of B. bacteriovorus cells. First, the ori locus was always located near the invasive, non-flagellated pole of G1 B. bacteriovorus cells, in contrast with the chromosomal orientation in previously characterized mono-flagellated bacteria.11,16,47,48 Based on RomR and flagellum labeling experiments, we conclude that the fraction of cells (~30%) in which ori and ter colocalized correspond to cases where ter, but not ori, is “misplaced.” Whether the occasional presence of ter at the invasive pole results from the relative flexibility of the ter macromdomain (reported in E. coli74,75) or from another aspect related to non-binary proliferation remains to be discovered.

Second, the relatively small area occupied by the nucleoid in G1 B. bacteriovorus cells indicates a dense chromosome meshwork. The unexpected non-homogeneous distribution of all freely diffusing fluorescent proteins that we tested showed partial exclusion from the DNA-containing region. So far, only larger objects were reported to be partially or fully excluded from the nucleoid (e.g., ribosomes or protein aggregates in E. coli61–65). Although these observations suggest that the compact DNA network constrains the mobility of small proteins in B. bacteriovorus, we cannot exclude that additional factors contribute to this phenomenon. Nevertheless, our observation that a free fluorescent protein is no longer nucleoid-excluded during the S phase is compatible with the idea of nucleoid condensation. This would be consistent with higher chromosome processing activities during growth (including DNA replication but also transcription70,71), which may remodel the nucleoid and/or require increased accessibility within the nucleoid. The dynamics of chromosome compaction and decompaction at the single-cell level, the architecture of the chromosome and the physiological impact of nucleoid condensation on the B. bacteriovorus cell cycle remain to be investigated.

Finally, we found that ParBbb is unable to accumulate at the ori region during the G1 and G1–S transition stages, even when produced constitutively. The detection of ParBbb foci after DnaN foci suggests that DNA replication initiation might open up the ori region, allowing ParBbb binding. It would be tempting to speculate that the accessibility of parSbb may vary during the cell cycle depending on the level of nucleoid compaction; however, the heterologous parSpmt1 inserted near endogenous parSbb sites remained accessible to ParBpm1 at all times. To the best of our knowledge, this is the first example of a ParB homolog that does not permanently localize at the chromosomal centromere, regardless of protein levels, hinting that beside transcriptional modulation70,71, ParBbb function could be subjected to novel cell-cycle-dependent regulation. Detailed mechanistic investigation of the ParABS system in Bdellovibrio, including the analysis of ParA subcellular dynamics, should reveal interesting features of this highly conserved partitioning machinery.

Altogether, our data illustrate that Bdellovibrio is a treasure-trove for future discoveries of novel cell-cycle regulation and cellular organization strategies. Moreover, our study sets the path for using B. bacteriovorus as a model to expand the quantitative investigation of subcellular events in bacteria and highlights the exploitation of conserved proteins to address the needs of complex non-binary reproduction.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCE TABLE**
  - **RESOURCE AVAILABILITY**
    - Lead contact
    - Materials availability
    - Data and code availability

- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Strains
  - Locus tags of genes used in this study
  - Routine culturing of B. bacteriovorus and E. coli

- **METHOD DETAILS**
  - Plasmid conjugation by mating
  - EdU labeling of newly synthesized DNA
  - Live-cell imaging
  - Image acquisition
  - Image processing
  - Western blot analysis
  - Killing curves
  - SYBR Green normalization of predator density

- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Cell, nucleoid and spot detection from images
  - Quantitative image analysis from cell meshes
  - Fluorescence profiles
  - Fluorescence spot position and intensity analysis
  - Nucleoid size measurement
  - Cell Projections using BactMAP
  - Killing curve analysis
  - Statistical analyses

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.06.024.
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STAR METHODS

KEY RESOURCE TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial strains** | See Table S1. | See Tables S1 and S3 | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Kanamycin | Sigma | #K4000 |
| Gentamycin | Sigma | #G1914 |
| Ampicillin | Sigma | #A9518 |
| Chloramphenicol | Sigma | #C0378 |
| Novobiocin | Sigma | #7467-1G |
| DAPI Nucleic Acid Stain | Life Technologies | #D1306 |
| SYTOX orange Nucleic Acid Stain | Life Technologies | #S11368 |
| Syto61 Nucleic Acid Stain | Thermo Fisher | #S11343 |
| FM4-64 Membrane Dye | Invitrogen | #T13320 |
| CellBrite™ Fix 488 Membrane Dye | VWR | #30090-T |
| Click-IT EdU Alexa Fluor Imaging Kit | Invitrogen | #C10337 |
| SYBR Green | Thermo Fisher | #4309155 |
| Deposited data | | |
| MATLAB codes and BactMAP cell projections workflow | This study | Zenodo: 10.5281/zenodo.4888934 |
| Oligonucleotides | See Table S4. | This study | N/A |
| Recombinant DNA | See Table S2. | This study | N/A |
| Software and algorithms | | |
| MATLAB | Mathworks | N/A |
| Oufiti | Paintdakhi:2016ex | http://oufiti.org/ |
| NIS-Elements Ar | Nikon Instruments Inc. | N/A |
| Fiji | Schindelin:2012ir | https://imagej.nih.gov/ij/ |
| Antibodies | | |
| Mouse monoclonal anti-GFP | Takara | #632380; RRID:AB_10013427 |
| Goat anti-mouse IgG-peroxidase | Sigma | #DC02L; RRID: AB_437851 |
| Rabbit polyclonal anti-mCherry | Thermo Fisher | #PA5-34974; RRID: AB_2552323 |
| Goat anti-rabbit IgG-peroxidase | Sigma | #A0545; RRID: AB_257896 |
| Other | | |
| Nikon T2-E inverted microscope | Nikon Instruments Inc. | N/A |
| Prime 95B sCMOS camera | Photometrics | N/A |
| Synergy H1m microplate reader | Biotek | N/A |

RESOURCE AVAILABILITY

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Géraldine Laloux (geraldine.laloux@uclouvain.be).

**Materials availability**
Plasmids and strains generated in this study will be shared by the lead contact upon request.
**Data and code availability**

Data reported in this paper will be shared by the lead contact upon request. All original Matlab codes used in this paper as well as the workflow to plot cell projections with BactMAP have been deposited at Zenodo (10.5281/zenodo.4888934) and are publicly available as of the date of publication. DOI is listed in the Key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Strains**

All strains and plasmids used in this study are listed in Table S1 and constructed as indicated in Tables S2 and S3. Standard molecular cloning methods were used, and DNA assembly was performed using the NEBuilder HiFi mix (New England Biolabs). All oligos used in this study are listed in Table S4. *B. bacteriovorus* strains were generated from the type strain HD100. *E. coli* strains used as prey were generated from MG1655. *E. coli* strains used for mating were generated from S17-λpir. All plasmids were introduced in *B. bacteriovorus* by mating as described below. Scarless allelic replacements into the HD100 chromosome were performed using a strategy based on the two-step recombination with pk18mobacB-derived suicide vector as in Steyert and Pineiro, resulting by PCR and verified by DNA sequencing. For homology, 500 bp upstream and downstream of the loci of interest were used. Protein fusions were confirmed by western blot (Figure S7B) and predation capacity of genetically engineered strains was verified by killing curves (Figure S7A), as described below.

**Locus tags of genes used in this study**

dnaN corresponds to Bd0002; dnaX corresponds to Bd3731; parBbb corresponds to Bd3905; romR corresponds to Bd2761 (old locus tags from the *B. bacteriovorus* HD100 genome annotation, NCBI Accession number NC_005363). The Bd0063-Bd0064 intergenic integration site is located between nucleotides 58,741 and 58,742.

**Labeling of ori and ter**

The *B. bacteriovorus* oriBb (further named ori) has been located between the dnaA and dnaN genes and the terBb region (ter) was identified by the 28-bp chromosome dimer resolution site dif, found between ORFs Bd2036 and Bd2038. The parSP1 and parSP2 sequences were inserted near these loci by allelic replacement (between ORFs Bd3895 and Bd3896, and between ORFs Bd2052 and Bd2053, i.e., ~17 kbp and 12 kbp away from oriBb and terBb, respectively. We chose insertion sites in non-coding regions of ~60 nucleotides between 3’ ends of predicted ORFs to avoid interrupting transcription initiation signals. The insertion sites between Bd3895 and Bd3896 (between 3,767,999 and 3,767,000), and between Bd2052 and Bd2053 (between 1,958,707 and 1,958,708) are referred to as ori and ter, respectively, for simplicity; the strains in which parSP1 or parSP2 was inserted at those loci are referred to as ori::parSP1 and ter::parSP2, respectively.

**Routine culturing of *B. bacteriovorus* and *E. coli**

*E. coli* cells were routinely grown in LB medium except when otherwise stated. *B. bacteriovorus* strains were grown in DNB medium (Dilute Nutrient Broth, Becton, Dickinson and Company, supplemented with 2 mM CaCl2 and 3 mM MgCl2 salts) with *E. coli* as prey at 30°C with constant shaking. Two-step revival of *B. bacteriovorus* from ~80°C stocks was performed as in Herencias et al., except that only DNB medium was used. When appropriate, antibiotic-resistant *E. coli* strains were used as prey for overnight culturing of the corresponding antibiotics-resistant *B. bacteriovorus*. Kanamycin and gentamycin were used at 50 μg/ml and 10 μg/ml, respectively, both in liquid and solid media.

**METHOD DETAILS**

**Plasmid conjugation by mating**

Mating was performed between *E. coli* S17-λpir donor strain carrying the plasmid to be conjugated and the *B. bacteriovorus* receiver strain using a protocol modified from Steyert and Pineiro. Briefly, exponentially growing donor strains were harvested and washed twice in DNB medium before resuspension in 1:10 of the initial volume in DNB-salts. This donor suspension was mixed at equal volume with a fresh overnight lysate of a receiver HD100 strain. The mating mix was incubated for minimum 4 h at 30°C shaking before plating on selective medium using the double layer technique. Single plaques were isolated and transconjugants were confirmed by microscopy (when appropriate), PCR and sequencing.

**EdU labeling of newly synthesized DNA**

Newly synthesized DNA in *E. coli* and *B. bacteriovorus* cultures was labeled using the Click-iT EdU Alexa Fluor Imaging Kit (Invitrogen, Germany) as performed before with other bacteria. Briefly, 200 μl of *B. bacteriovorus* cells grown exponentially in M9-glucose medium were incubated with ~12 μM 5-ethynyl-2'-deoxyuridine (EdU) for 5 and 15 minutes, respectively. Cells were fixed with 78% of ice-cold methanol to stop the reaction, washed in PBS (5000 x g, 4°C, 5 min), before membrane permeabilization in 100 μl PBS containing 0.5% Triton X-100 at room temperature for 30 minutes. Hereafter, the detergent was washed off twice with PBS. The pellet was resuspended in 40 μl of Click-iT reaction cocktail and incubated at room temperature.
covered from light for 30 minutes. The cells were collected, washed, resuspended in 40 μl of PBS, and when required treated with 5 μg/ml DAPI before imaging.

**Live-cell imaging**

*B. bacteriovorus* were first grown overnight with the appropriate *E. coli* prey and antibiotics if maintenance of a plasmid was required, then grown on wild-type MG1655 for at least one generation without antibiotic before the start of the imaging experiment. For snapshots of fresh AP *B. bacteriovorus*, cells were then spotted on 1.2% agarose pads prepared in DNB-salt media. For snapshots of *E. coli* strains, overnight cultures were diluted at least 1:500 and grown to exponential phase before being spotted on 1.2% agarose pads prepared in PBS or M9-salts buffer (supplemented with 0.2% glucose, 0.2% casamino acids and 1 μg/ml thiamine, 2 mM MgSO4 and 0.1mM CaCl2). For time-lapse or time-course imaging of synchronous predation cycles, MG1655 *E. coli* cells were grown in 2TYE medium to exponential phase (OD600 = 0.4-0.6), harvested at 2600 x g at RT for 5 minutes, washed twice and resuspended in DNB medium. Then, *E. coli* and *B. bacteriovorus* were mixed with a 1:3 to 1:5 volume ratio to allow most prey cells to be infected simultaneously. We consider the prey-predator mixing step as the time 0 in all our synchronous predation imaging experiments. Cells were either spotted directly on DNB-agarose pads for imaging, or left shaking at 30°C before imaging for the indicated durations. In time-lapse experiments, the same fields of view on the pad were imaged at regular interval times as indicated, with the enclosure temperature set to 28°C or 30°C. In time-course experiments, samples from the predation mixture were taken at regular interval times as indicated and directly spotted on agarose pads for snapshots. For nucleoid staining experiments, cells were incubated for 5 min prior imaging with DAPI (Life Technologies), SYTOX orange (Life Technologies) or Syto61 (Thermo Fisher) at a final concentration of 20 μg/ml, 500 nM and 200 nM, respectively. For flagellum staining, *B. bacteriovorus* AP cells were stained with the FM4-64 stain (Thermo Fisher) at a final concentration of 20 μg/ml and incubated in the dark for 2 min before detection or with CellBrite™ Fix 488 Membrane Dye (VWR) at a final concentration of 10X (from a 1:1000 dilution) and incubated in the dark for 2 min before detection. For treatment with novobiocin, fresh AP *B. bacteriovorus* cells were mixed with prey as explained above, treated or not with 5 μg/ml novobiocin (Sigma) at the indicated times and before being immediately spotted on agarose pads containing 5 μg/ml novobiocin or not, respectively.

**Image acquisition**

Phase contrast and fluorescence images were acquired on a Nikon Ti2-E fully-motorized inverted epifluorescence microscope (Nikon) equipped with CFI Plan Apochromat 40X DM 100x 1.45/0.13 mm Ph3 oil objective (Nikon), a Sola SEII FISH illuminator (Lumencor), a Prime958 camera (Photometrics), a temperature-controlled light-protected enclosure (Okolab), and filter-cubes for DAPI, CFP, mCherry, YFP and GFP (Nikon). Multi-dimensional image acquisition was controlled by the NIS-AR software (Nikon). Pixel size was 0.11 μm or 0.07 μm when using built-in 1X or 1.5X intermediate magnification, respectively. Identical LED illumination and exposure times were applied when imaging several strains and/or conditions in one experiment and were set to the minimum for time-lapse acquisitions to limit phototoxicity.

**Image processing**

For figure preparation, images were processed with FIJI® keeping contrast and brightness settings identical for all regions of interest in each figure, except when otherwise stated. For Figures 4A, 5C, 6A, S5A, and S5B denoising (Denoise.ai, Nikon) was applied on all figures. Image processing was performed using standard procedures with the following primary antibodies: JL-8 monoclonal antibody (Takara) for GFP variants, YFP and CFP; polyclonal mCherry antibody (product # PA5-34974, Thermo Fisher) for mCherry. Signal from antibody binding was visualized by detecting chemiluminescence from the reaction of horseradish peroxidase with luminol and chemiluminescence was imaged with an Image Quant LAS 500 camera (GE Healthcare). Goat anti-mouse IgG-peroxidase antibody (Sigma) was used as a secondary antibody for JL-8. Goat anti-rabbit IgG-peroxidase antibody (Sigma) was used as a secondary antibody for mCherry. Antibodies were diluted following manufacturer’s recommendations. Figures were prepared using ImageJ and assembled and annotated using Adobe Illustrator.

**Western blot analysis**

Sample preparation for western blot analysis was performed as in Denoncin et al., starting from 3 mL in the case of cleared *B. bacteriovorus* lysates. Sample were loaded on NuPage Bis-Tris SDS precast polyacrylamide gels and ran at 190 V for 50 minutes in NuPAGE MES SDS running buffer. Western blotting was performed using standard procedures with the following primary antibodies: JL-8 monoclonal antibody (Takara) for GFP variants, YFP and CFP; polyclonal mCherry antibody (product # PA5-34974, Thermo Fisher) for mCherry. Signal from antibody binding was visualized by detecting chemiluminescence from the reaction of horseradish peroxidase with luminol and chemiluminescence was imaged with an Image Quant LAS 500 camera (GE Healthcare). Goat anti-mouse IgG-peroxidase antibody (Sigma) was used as a secondary antibody for JL-8. Goat anti-rabbit IgG-peroxidase antibody (Sigma) was used as a secondary antibody for mCherry. Antibodies were diluted following manufacturer’s recommendations. Figures were prepared using ImageJ and assembled and annotated using Adobe Illustrator.

**Killing curves**

Killing curves assays (Figure S7A) were performed after normalization of the *B. bacteriovorus* inoculum (using the SYBR Green assay described below). Equal amounts of predators from the same fresh cleared lysate were mixed with preys at a final OD600 of 0.1, and DNB medium was added to reach 150 μl per well in a transparent 96-well flat bottom plate. Technical triplicates were prepared in separate wells of the same plate in each experiment. The plate was shaken continuously (frequency 567 cpm (3mm)) at 30°C for 24 h in a Synergy H1m microplate reader (Biotek). Optical density measurements at 600 nm were taken every 20 minutes. Decrease of OD600 indicates prey lysis, as *B. bacteriovorus* cells do not affect absorbance.
SYBR Green normalization of predator density
For each cleared lysate of _B. bacteriovorus_ to analyze, 198 μl/well were transferred into 3 wells of a black 96-well plate with transparent flat bottom (Sigma Aldrich). Then, protected from light, 2 μl of SYBR Green (LifeTechnologies) were added to each replicate to reach a volume of 200 μl per well. Plates without lid were shaken (double orbital, frequency 282 cpm (3 mm)) in a Synergy H1m microplate reader (Biotek), for 15 min at 25˚C before one end-point measurement of both OD600 and the SYBR Green fluorescence (490 nm excitation, 520 nm emission, gain 55). Based on a standard curve of OD600 relative to fluorescence values (obtained from serially diluted _E. coli_ suspensions), the contribution of remaining _E. coli_ in the lysates to the measured fluorescence was subtracted from the total SYBR Green fluorescence value in each well. The mean of the corrected fluorescence values from the 3 replicates is then used to compare the _B. bacteriovorus_ density in different lysates and normalize them accordingly.

QUANTIFICATION AND STATISTICAL ANALYSIS

Cell, nucleoid and spot detection from images
Cell outlines were obtained with subpixel precision from phase contrast images for AP _B. bacteriovorus_ cells, uninfected _E. coli_ cells or entire bdelloplasts using the automated cellDetection tool in the open-source image segmentation and analysis software Oufti. For analysis of intracellular signal in _B. bacteriovorus_ cells within bdelloplasts, predator cell outlines were manually added in Oufti using the same parameters optimized on the appropriate control set of images.

Kymographs and demographs
Demographs of relative fluorescence intensity in cells sorted by length were plotted as in Paintdakhi et al. and Sliusarenko et al. When needed, arrays of relative fluorescence were oriented based on the position of the maximal fluorescence intensity of the indicated signal in each cell half. Kymographs were obtained using the built-in kymograph function in Oufti.

Quantitative image analysis from cell meshes
Fluorescence-related analysis, nucleoids and spots-related information, as well as other properties of individual cells based on microscopy images were extracted from Oufti data and plotted using custom codes in MATLAB (Mathworks), described below.

Fluorescence profiles
To obtain mean relative fluorescence profiles (MeanIntProfile.m), the fluorescence profile of each cell (corresponding to the array of fluorescence intensity provided by the relevant signal field in the Oufti cellList) was first normalized by the corresponding steparea values (normalization through cellular concentration), then divided by their sum to obtain relative fluorescence values for each cell (to account for potential concentration differences between cells). When needed, arrays of relative fluorescence were oriented based on the position of the maximal fluorescence intensity of the indicated signal in each cell half. Cell length vectors were normalized from 0 to 1 and the corresponding relative fluorescence profiles were interpolated to a fixed dimension vector and concatenated before averaging. Fluorescence intensity profiles along the centerline of individual cells (Figures 4B, S3D, and S4C) were obtained by plotting linescans from segmented lines drawn across each cell in FIJI.

Fluorescence spot position and intensity analysis
To obtain spot-to-pole distances (histSpotsDistPole.m), the position of single spots along the cell center line was normalized by cell length to obtain relative position values, which were subtracted from 1 if higher than 0.5 in order to obtain relative positions from pole (0) to midcell (0.5). Histograms were then computed and plotted as lines in MATLAB (Mathworks) using built-in functions. Only cells with one spot were kept for this analysis. To obtain the spot/cytosol fluorescence ratio (RatioSpotFluoVsCellFluo.m), the relative fluorescence intensity (i.e., total fluorescence divided by area) was calculated for (i) a region of the cell containing the spot, which is defined as the segment where the spot is centered ± 2 segments, and (ii) the cell body, which corresponds to the rest of the cell (i.e., excluding the spot region). The script then computes the ratio of relative fluorescence in the spot versus in the cell body. Fluorescent spots were considered as colocalized in AP cells when the distance between the center of each spot was below a threshold of 200 nm (colocSpots2signals.m). Colocalization was evaluated manually for cells inside bdelloplasts.
**Nucleoid size measurement**

We considered the nucleoid area obtained after running the objectDetection module in Oufti as a proxy for nucleoid size, based on Gray et al., which showed using a similar detection method that nucleoid area measurements are unaffected by variations in DAPI signal intensity. Nucleoid area is provided by the object.area field in the Oufti cell lists, and values were collected in MATLAB for all cells with a single nucleoid and converted to μm². Nucleoid area distributions were plotted in MATLAB except violin plots in Figure 2D, which were plotted on R.

**Cell Projections using BactMAP**

The development version of BactMAP (https://github.com/vrrenske/BactMAP) was used to generate cell projections (Figure S3B). The function orientCells() was written to orient cells by shape and subsequently by their fluorescent focus; cells without focus or with more than one focus were removed from the analysis. After this, the BactMAP function plotOverlay() was used to group cells by cell length into four equally-sized groups and plot the cell shape, DAPI shape and fluorescent spot localization of each cell, faceted by size group.

**Killing curve analysis**

Features of the curves were extracted by fitting the data to an adaptation of the generalized sigmoid curve (Equation 1) using an R workflow based on a differential evolution algorithm. Briefly, a first rough fit is done using all the data to find an approximation of the sigmoid midpoint. s is then used to estimate the sigmoid part of the data on which a second, more accurate fit is performed. Data were plotted using R programming language.

\[ P(t) = \frac{P_{\text{max}} - P_{\text{min}}}{1 + e^{\frac{s - t}{P_{\text{max}} - P_{\text{min}}}}} \]  
(Equation 1)

**Statistical analyses**

The sample sizes and number of repeats are included in the figure legends. Means, standard deviations and coefficients of variation (CV) were calculated in MATLAB (Mathworks) or Microsoft Excel. SuperPlots were generated in Microsoft Excel as described.
Supplemental Information

Chromosome choreography
during the non-binary cell cycle
of a predatory bacterium

Jovana Kaljević, Terrens N.V. Saaki, Sander K. Govers, Ophélie Remy, Renske van Raaphorst, Thomas Lamot, and Géraldine Laloux
A

Number of foci per cell

Fraction of cells (%)

0 1 2

YFP-ParB

PMT1

n = 3266

n = 4469

n = 3593

B

CFP-ParB

~17kb

~8.3kb

ori::parS

PMT1

ter::parS

P1

C

Phase

YFP-ParB

PMT1

n = 3814

n = 5119

n = 3593

n = 4459

YFP-ParB

PMT1

CFP-ParB

P1

ori::parS

PMT1

Control (no parS

PMT1)

n = 2564

n = 3814

n = 3745

n = 5767
Figure S1. Specificity of the orthologous parS-ParB labelling system. Related to Figure 1. (A) Histograms representing the percentage of cells with zero, one or two YFP-ParB<sub>PMT1</sub>, or CFP-ParB<sub>P1</sub> foci per cell in strains ori::parS<sub>PMT1</sub>/pTNV215-yfp-parB<sub>PMT1</sub>, ter::parS<sub>P</sub>/pTNV215-cfp-parB<sub>P1</sub> and ori::parS<sub>PMT1</sub>ter::parS<sub>P1</sub>/pTNV215-yfp-parB<sub>PMT1</cfp-parB<sub>P1</sub> (GL868, GL771 and GL995, respectively). All foci were observed at the poles. The small fractions of cells without focus failed to match stringent spot detection parameters in Oufti. (B) Schematic overview of the orthologous parS-ParB pairs as in Fig 1B and controls. From left to right: only specific interactions between the corresponding pairs form a clear fluorescent focus after imaging; schematics of the relative genomic positions of ori<sub>Bb</sub> (pink), endogenous parS<sub>Bb</sub> (grey) and integrated orthologous parS<sub>PMT1</sub> (yellow). (C) Control experiments for parS-ParB specific binding. Left to right (from the top): representative phase contrast and fluorescence images of AP cells of strain ter::parS<sub>P1</sub> expressing non-cognate YFP-ParB<sub>PMT1</sub> (GL772); histograms of cells with zero, one or two YFP-ParB<sub>PMT1</sub> foci; profile as in Fig 1C; distribution of the ratio of YFP-ParB<sub>PMT1</sub> fluorescence intensity in the spot vs in the cytosol measured in cells with one detected focus (orange line: GL772, black line: GL868). As above for cells expressing YFP-ParB<sub>PMT1</sub> in a wild-type (WT) background (strain GL785); orange line: GL785, black line: GL868. Images and profile as above for strain ori::parS<sub>PMT1</sub> expressing non-cognate CFP-ParB<sub>P1</sub> (GL867), which localizes on the nucleoid. As above for cells expressing CFP-ParB<sub>P1</sub> in a wild-type (WT) background (GL784). Images, histogram, and profiles for the indicated fusions for ori::parS<sub>PMT1</sub> cells expressing non-cognate CFP-ParB<sub>P1</sub> and cognate YFP-ParB<sub>PMT1</sub> (GL869). Representative phase contrast and fluorescence images of AP cells of strain ter::parS<sub>P1</sub> expressing cognate CFP-ParB<sub>P1</sub> and non-cognate YFP-ParB<sub>PMT1</sub> (GL773); orange line in the ratio distribution plot: GL773, black line: GL868. Schematics illustrate the ori and ter labelling construct used in each panel. Scale bars are 1 µm. n indicate the number of cells analysed in a representative experiment. Experiments were performed at least twice. For histograms: using the same parameters for automated spot detection as in Fig 1C, YFP-ParB<sub>PMT1</sub> spots were detected in a lower fraction of control cells lacking the cognate parS<sub>PMT1</sub>. Analysis showed that these spots are distinct from the foci seen in strain GL868 (which carries parS<sub>PMT1</sub>), supporting the idea that polar accumulations in control strains are non-specific: (1) mean oriented profiles of relative YFP-ParB<sub>PMT1</sub> fluorescence intensity show less difference between the region containing the highest fluorescence and the rest of the cell, compared to the profile in Fig 1C for strain GL868, and (2) distributions of the ratio of YFP-ParB<sub>PMT1</sub> fluorescence intensity in the spot vs in the cytosol show that spots detected in GL868 (black line) have higher intensity than spots in control strains (orange lines). Of note, CFP-ParB<sub>P1</sub> produced in the absence of its cognate parS<sub>P1</sub> sequence appears nucleoid-bound. All cell outlines were obtained with Oufti.
Figure S2. Localization of ori and ter markers with lower ParB-FP levels, and localization of ori and ter markers during prey attachment and upon flagellum staining. Related to Figure 1. (A-B) ParBPMT1 and ParBP1 fusions expressed from a Bdellovibrio promoter are produced at low levels and label ori::parSPMT1 and ter::parSP1 only in attack phase. (A) Distributions of mean fluorescence intensity values for cells with one detected fluorescent spot: left: AP cells of ori::parSPMT1 strains expressing cognate YFP-ParBPMT1 from PBd3471 (GL1476, red) or the constitutively active promoter PnptII (GL868, yellow); right: AP cells of ter::parSP1 strains expressing cognate CFP-ParBP1 from the PBd3471 promoter (GL1475, red) or PnptII promoter (GL771, cyan); representative images are shown. (B) Representative phase contrast and fluorescence images for the same cells as in A during growth phase (GP). Cells were imaged 3 h after mixing with prey. Arrowheads point to foci in AP B. bacteriovorus cells (in contrast with the absence of signal in the GP cell on the same image). (C) RomR is a marker of the invasive pole. Representative phase contrast and fluorescence images of AP cells of cells constitutively producing cognate RomR-TdTomato from a plasmid in WT background (strain GL512) prior labelling with CellBrite™ Fix 488 membrane dye. Arrowhead points to the RomR-tdTomato polar focus. (D) As in Figure 1F for ori::parSPMT1 and ter::parSP1 strains producing cognate YFP-ParBPMT1 or CFP-ParBP1 (strains GL868 and GL771, respectively). (E) Representative phase contrast and fluorescence images of strains GL868, GL1476, GL771 and GL1475 after staining with FM4-64 (see genotypes in legend of panel A); the tagged loci and promoters controlling the expression of corresponding ParB fusions are indicated; arrowheads point to polar foci. (F) Same as in Figure 1G for the ori::parSPMT1 ter::parSP1 strain constitutively producing cognate CFP-ParBP1 and YFP-ParBPMT1 from the PnptII promoter (strain GL995); the top and bottom parts represent the most and least frequent configurations, respectively (fraction of cells with colocalized ori and ter is indicated). Scale bars are 1 μm. n indicate the number of cells analysed manually in a representative experiment. All outlines of bdelloplasts were drawn manually based on phase contrast images; All cell outlines of AP Bdellovibrio cells were obtained with Oufti.
Figure S3. Nucleoid compaction in *B. bacteriovorus*. Related to Figure 2 and Figure 3. (A) Left to right: representative phase contrast and fluorescence images of AP cells of WT *B. bacteriovorus* stained with SYTOX orange; demograph of the corresponding fluorescent signal in the same cells. Heatmaps represent relative fluorescence intensities. (B) Key chromosomal loci, ori and ter localize at the nucleoid tips. Images shown in Figure 2C. Demograph of the corresponding fluorescent signals in the same cells, oriented based on signal intensity of the indicated ParB fusion. Heatmaps represent relative fluorescence intensities. (C) Projections where cells were grouped by cell length and cell shape, each projected stack of cells including DNA-staining shape and fluorescent spots as indicated. Bottom right: histograms of cell lengths for WT (HD100), ori::parS <sup>PMT1</sup>/pTNV215-yfp-<sup>parB</sup><sub>PMT1</sub> (GL868) and ter::parS<sub>P</sub>/pTNV215-cfp-<sup>parB</sup><sub>P1</sub> (GL771) strains; mean and standard deviation values are shown; n indicate the number of cells analysed for each strain in a representative experiment. Schematics illustrate the ori and ter labelling constructs used in this panel. (D) Partial nucleoid exclusion of free fluorescent proteins. Representative phase contrast and fluorescence images of strains *Bd0063-0064::pBioFa*-<sup>b</sup>-<sup>sf</sup><sub>tq2ox</sub> and *Bd0063-0064::pBioFab-mcherry* (GL1024 and GL1025, respectively), or WT/pTNV215-<sup>msfgfp</sup> (GL1208) stained with SYTOX orange or DAPI as indicated. Arrowheads point to nucleoid exclusions on an enlarged example (inset). Scale bar is 1 µm except for enlarged examples where scale bar is 0.5 µm. (E) Fluorescence intensity profiles of the corresponding signals in representative cells from D and Figure 2E. (F-H) The nucleoside analogue EdU marks areas within the cell where new DNA is being synthesized, positive and negative controls in *E. coli*. (F) Representative overlays of phase contrast and fluorescence images of WT strain exposed to a 15-minute pulse of EdU and Alexa488 in different combinations; arrows point to foci of Alexa488-labeled EdU; no Alexa488 foci were observed in the absence of EdU. (G) WT and *seqA::seqA-mCherry* strains, exposed to a 15-minute pulse of EdU, which was fluorescently labelled with Alexa488 and stained with DAPI when indicated. EdU-Alexa488 and SeqA-mCherry foci colocalize. (H) The nucleoside analogue EdU marks areas within the cell where new DNA is being synthesized in *B. bacteriovorus*. Representative overlays of phase contrast and fluorescence images of WT and *parB<sub>Bb</sub>::parB<sub>Bb</sub>-mCherry* (GL906) strains 150 min after mixing with prey, in both cases, and exposed to a 5-minute pulse of the EdU and Alexa488 in different combinations; arrows point two foci of Alexa488-labeled EdU; arrowheads point to the colocalization of one focus of EdU, which was fluorescently labelled with Alexa488, and one or two ParB<sub>Bb</sub>-mCherry, respectively which mark the position of oriC. Scale bar is 2 µm, applicable to all panels. For all, cell outlines of AP *Bdellovibrio* cells were obtained with Oufiti.
Figure S4. Replisome dynamics and nucleoid localization in *B. bacteriovorus*. Related to Figure 4. (A) Localization dynamics of DnaX in *B. bacteriovorus*. *B. bacteriovorus* strain *dnaX::dnaX-msfGFP* (GL1364) was mixed with prey and imaged in time-course every 30 min after mixing with prey; phase contrast and fluorescence images of selected timepoints from a representative experiment are shown. Scale bar is 1 µm. (B) Gyrase inhibitor novobiocin prevents new rounds of DNA replication initiation. *B. bacteriovorus* strain *dnaN::dnaN-msfGFP* (GL673) was mixed with prey, not treated (-) or treated (+) with 5 µg/ml novobiocin (top) 65 min after mixing with prey or (bottom) 155 min after mixing with prey, before time-lapse imaging at 5 min intervals on agarose pads containing 5 µg/ml novobiocin (+) or not (-). Phase contrast and fluorescence images of selected timepoints of a representative experiment are shown. Inhibitory effect is indicated by a disassembly of the existing DnaN-msfGFP foci and the absence of new foci formation. Scale bar is 1 µm. (C) DAPI staining in cells with labelled replisome. *B. bacteriovorus* strain *dnaN::dnaN-msfGFP* (GL673) was mixed with prey and imaged in time-course at 30 min intervals. Top: phase contrast and fluorescence images of selected timepoints from a representative experiment are shown; arrowheads point to regions with less DAPI signal and where replisomes are located. Scale bar is 1 µm. For all, outlines of *B. bacteriovorus* and bdelloplasts were drawn manually based on phase contrast images. Bottom: fluorescence intensity profiles of the corresponding signals in the same cells; arrowheads point to regions with less DAPI signal and where replisomes are located.
Figure S5. Spatio-temporal arrangement of the chromosome during the S phase and ParB<sub>Bb</sub> localization in AP cells. Related to Figure 5. (A) After a first asymmetric segregation, additional ori foci appear. <i>B. bacteriovorus</i> strain ori::parSP<sub>M1</sub> expressing cognate YFP-ParB<sub>PMT1</sub> (GL868) was mixed with prey and imaged in time-course with 30 min intervals. Phase contrast and fluorescence images of selected timepoints of a representative experiment are shown; arrowheads point to fluorescent foci. (B) ter dynamics during the proliferative phase. <i>B. bacteriovorus</i> strain ter::parSP<sub>1</sub> expressing cognate CFP-ParB<sub>P1</sub> (GL771) was mixed with prey and imaged in time-course with 30 min intervals (left) or time-lapse after 90 min with 7 min intervals (right). For each, phase contrast and fluorescence images of selected timepoints of a representative experiment are shown; arrowheads point to polar then mid-cell ter localization; asterisks point to evenly distributed ter copies; timepoint 461 min from time-lapse illustrates ter foci disassembly; circles point to re-appearance of ter foci in daughter cells. (C) Endogenous ParB<sub>Bb</sub> does not form an ori-bound focus during AP. Representative phase contrast and fluorescence images of attack phase cells of parB<sub>Bb</sub>::parB<sub>Bb</sub>-mcherry strain (GL906) stained with DAPI; histogram of mean fluorescence intensity of the corresponding signal in the same cells; mean mCherry fluorescence intensity in the same GL906 cells compared to WT; n indicate the number of cells analysed in a representative experiment; mean values are represented. Error bars indicate standard deviations. Cell outlines were obtained with Oufti. (D) Endogenous ParB<sub>Bb</sub> forms a focus that colocalizes with the replisome at the start of the S phase. Representative phase contrast and fluorescence images of dnaN:: dnaN-msf GFP parB<sub>Bb</sub>::parB<sub>Bb</sub>-mcherry strain (GL1055) imaged 110 min after mixing with <i>E. coli</i> prey; colocalization in 93% (n=111 from one representative experiment, colocalization was quantified manually). (E) ParB<sub>Bb</sub>-mCherry is bona fide marker of ori in <i>Bdellovibrio bacteriovorus</i>. <i>B. bacteriovorus</i> strain parB<sub>Bb</sub>::parB<sub>Bb</sub>-mcherry ori::parSP<sub>M1</sub> expressing cognate YFP-ParB<sub>PMT1</sub> (GL1367) was mixed with prey and imaged in time-course with 30 min intervals. Top: phase contrast and fluorescence images of selected timepoints from a representative experiment are shown; both signals colocalized through the cycle (pink and yellow arrowheads), except in the early stages where only signal from YFP-ParB<sub>PMT1</sub> was visible (yellow asterisk). Bottom: mean pole-to-pole profiles of relative fluorescence intensity of the corresponding fusions in the same cells. Schematics illustrate the ori and ter labelling constructs used in appropriate panels. Scale bars are 1 µm. For all, except in C, outlines of <i>B. bacteriovorus</i> and bdelloplasts were drawn manually based on phase contrast images.
Figure S6. The ParABS system contributes to progressive ori segregation. Related to Figure 6. (A) Foci formed by overproduced ParB\textsubscript{Br}-mCherry label ori. Top: time-course experiment of an ori::parS\textsubscript{PMT1} strain constitutively expressing YFP-ParB\textsubscript{PMT1} and ParB\textsubscript{Br}-mCherry (GL1372); both signals colocalized through the cycle (pink and yellow arrowheads), except in the early stages where foci were visible only for YFP-ParB\textsubscript{PMT1} (yellow arrowhead); cells were mixed with prey and imaged at 30 min intervals; selected frames are presented. Bottom: mean pole-to-pole profiles of relative fluorescence intensity of the corresponding fusions in the same cells. n indicate the number of cells analysed in a representative experiment. (B) Overproduction of ParB\textsubscript{Br} leads to phenotypic changes in AP cells. Representative phase contrast and fluorescence images of AP cells of WT strains constitutively expressing ParB\textsubscript{Br}-mCherry (top) and ParB\textsubscript{Br}-msfGFP (bottom) stained with DAPI (GL1002 and GL1003, respectively). (C) Overexpression of ParB\textsubscript{Br} leads to pronounced phenotypes. Histograms of cell length, cell area, nucleoid area and NC ratio for the cells shown in B; mean and standard deviation values are shown. (D) Overexpression of ParB\textsubscript{Br} leads to aberrant numbers of ori copies in AP cells. From left to right: representative phase contrast and fluorescence images of AP cells of the same strain shown in A (GL1372) stained with DAPI; arrowheads point to ori foci; histograms representing the proportion of cells with zero, one or two YFP-ParB\textsubscript{PMT1} foci in the same cells; relative pole to mid-cell distance profile for YFP-ParB\textsubscript{PMT1} in the same cells. Fluorescent signal from ParB\textsubscript{Br}-mCherry is not shown for simplicity, both signals always colocalized (see A). Schematics illustrate the ori labelling construct. (E) Partial nucleoid exclusion of cytoplasmic fluorescent proteins in ParB\textsubscript{Br} overproducing strain. Representative phase contrast and fluorescence images of strain Bd0063-0064::pBioFab-mcherry/pTNV215-parB\textsubscript{Br}-msfgfp (GL1388) stained with DAPI. Arrowheads point to nucleoid exclusions on an enlarged example (inset). (F) Dynamics of chromosome replication. SuperPlot representation of the time of appearance of first and second DnaN-msfGFP focus in strain dnaN::dnaN-msfgfp (GL673) (left), and the time difference between appearance of first and second DnaN-msfGFP foci in the same cells (right). (G) Dynamics of chromosome segregation. SuperPlot representation of the time of appearance of first and second ParB\textsubscript{Br}-mCherry focus in strain parB\textsubscript{Br}::parB\textsubscript{Br}-mcherry (GL906) (left), and the time difference between appearance of first and second ParB\textsubscript{Br}-mCherry foci in the same cells (right). (F-G) Each biological replicate is color-coded; the average from each replicate is represented as a colored diamond and the mean values obtained from all replicates averages are represented as bars. Calculated values are summarized in the corresponding tables; n indicate the number of cells analyzed in each experiment. Scale bars are 1 µm except for enlarged examples where scale bar is 0.5 µm. Outlines of B. bacteriovorus and bdelloplasts were obtained with Oufti, except in A where they were drawn manually based on phase contrast images. Experiments were performed at least twice.
Figure S7. Killing rates of *B. bacteriovorus* strains and Western Blot detection of protein fusions used in this study. Related to STAR Methods. (A) Left: killing curves of *WT* cells (cyan) and various *B. bacteriovorus* strains (red). *k* indicates the mean killing rate (h⁻¹) calculated for each strain based on biological replicates (i.e. predator lysates coming from distinct plaques) shown on the graphs, from one representative experiment out of at least two independent repeats. Representative experiments are colour-coded. Right: mean and standard deviations of killing rates (h⁻¹) corresponding to data shown on the left. (B-C) Western blots of whole-cell protein extracts from *B. bacteriovorus* and *E. coli* were probed with α-GFP (B) and α-mCherry (C) antibodies to confirm proper protein production. (B) Lanes 1-15 are for (1) ter::parS₁/pTNV215-cfp-parB₁ (GL771), (2) WT/pTNV215-cfp-parB₁ (GL784), (3) ter::parS₁/pTNV215-yfp-parB₁ (GL772), (4) ter::parS₁/pTNV215-yfp-parB₁-cfp-parB₁ (GL773), (5) WT/pTNV215-yfp-parB₁ (GL785), (6) ori::parS₁/pTNV215-cfp-parB₁ (GL867), (7) ori::parS₁/pTNV215-yfp-parB₁-cfp-parB₁ (GL869), (8) ori::parSMT1 ter::parS₁/pTNV215-cfp-parB₁-cfp-parB₁ (GL995), (9) Bd0063-0064::pBioFab-sftq2ox (GL1024), (10) WT/pTNV215-msfgfp (GL1208), (11) MG1655/pBAD18-msfgfp (GL726), (12) dnaN::dnaN-msfgfp (GL673), (13) ori::parSMT1/pTNV215-yfp-parB₁ (GL868), (14) WT/pTNV215-parB₅ₐ⁻msfgfp (GL1003), (15) Bd0063-0064::pBioFab-sfgfp (GL1212). (C) Lanes 1-3 are for: (1) Bd0063-0064::pBioFab-mcherry (GL1025), (2) WT/pTNV215-parB₅ₐ⁻mcherry (GL1002), (3) MG1655/pBAD18-mcherry (GL727). *B. bacteriovorus* wild-type controls are shown: GL734 (*WT₁*) and GL499 (*WT₂*). Detected proteins and their expected sizes are indicated for each panel. Asterisks are on top or next to full-length protein fusions when non-specific bands are present. Ponceau staining of the same membranes (where bands were most visible, ~30-50 kDa) is illustrated below each blot as a loading indicator. Molecular weight markers (kDa) are shown on the side.
| Strains     | Description                                             | Resistance | Source or reference     |
|------------|---------------------------------------------------------|------------|-------------------------|
| GL499      | HD100                                                   | /          | ATCC strain 15356       |
| GL512      | HD100 / pMQ414-romR                                      | Gm         | This study              |
| GL734      | HD100                                                   | /          | S1 (kind gift from R.E. Sockett, U. Nottingham) |
| GL673      | HD100 dnaN::dnaN-msfgfp                                  | /          | This study              |
| GL676      | HD100 ori::parSP1 ter::parSP1                           | /          | This study              |
| GL771      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL772      | HD100 ori::parSP1 ter::parSP1 tDNA-romR                 | Gm         | This study              |
| GL773      | HD100 ori::parSP1 ter::parSP2 tDNA-romR                 | Gm         | This study              |
| GL784      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL785      | HD100 / pTNV215-yfp-parB101                              | Gm         | This study              |
| GL806      | HD100 ori::parSP1 ter::parSP1                           | /          | This study              |
| GL816      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL867      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL868      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL869      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL870      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL906      | HD100 parB101::parB101 mcherry                          | /          | This study              |
| GL909      | HD100 ori::parSP1 ter::parSP1                           | /          | This study              |
| GL995      | HD100 ori::parSP1 ter::parSP1                           | Gm         | This study              |
| GL1002     | HD100 / pTNV215-parB101 mcherry                         | Gm         | This study              |
| GL1003     | HD100 / pTNV215-parB101 mcherry                         | Gm         | This study              |
| GL1024     | HD100 Bd0063-0064::pBioFab-sfg20x                        | /          | This study*             |
| GL1025     | HD100 Bd0063-0064::pBioFab-sfg20x                        | /          | This study              |
| GL1055     | HD100 parB::parB101 mcherry dnaN::dnaN-msfgfp            | /          | This study              |
| GL1102     | HD100 ori::parSP101 dnaN::dnaN-msfgfp                    | /          | This study              |
| GL1103     | HD100 ori::parSP101 dnaN::dnaN-msfgfp / pTNV215-mCherry-parB101 | Gm | This study              |
| GL1121     | HD100 parB::parB101 mcherry ori::parSP101               | /          | This study              |
| GL1122     | HD100 parB::parB101 mcherry ter::parSP101               | /          | This study              |
| GL1208     | HD100 / pTNV215-msfgfp                                  | Gm         | This study              |
| GL1211     | HD100 dnaN::dnaN-msfgfp / pTNV215-romR-ttdtomato         | Gm         | This study              |
| GL1212     | HD100 Bd0063-0064::pBioFab-sfgfp                         | /          | This study              |
| GL1261     | HD100 / pTNV215-parB101                                 | Gm         | This study              |
| GL1364     | HD100 dnaX::dnaX-msfgfp                                  | /          | This study              |
| GL1367     | HD100 parB::parB101 mcherry ori::parSP101               | /          | This study              |
| GL1368     | HD100 parB::parB101 mcherry ter::parSP101               | Gm         | This study              |
| GL1372     | HD100 ori::parSP101 ter::parSP101                       | Gm         | This study              |
| GL1388     | HD100 Bd0063-0064::pBioFab-mcherry / pTNV215-parB101 msfgfp | Gm | This study              |
| GL1475     | HD100 ter::parSP1 / pTNV215-PBd3471-cfp-parB101         | Gm         | This study**             |
| GL1476     | HD100 ori::parSP1 / pTNV215-PBd3471-cfp-parB101         | Gm         | This study              |
### E. coli

| Strains | Description | Resistance | Source or reference |
|---------|-------------|------------|---------------------|
| S17-1 λpir | Donor strain for conjugative transfer (chromosomally integrated RP4 plasmid) | Strep | Lab collection |
| MG1655 | WT E. coli strain used as prey for B. bacteriovorus | / | Lab collection |
| GL503 | S17-1 λpir / pXDB013 (pMR-ylp-parB<sub>PM1</sub>-<sub>Pr</sub>-parB<sub>pr1</sub>) | Kan | s2 |
| GL504 | S17-1 λpir / pXDB014 (pMR-ylp-parB<sub>PM1</sub>) | Kan | s2 |
| GL505 | S17-1 λpir / pXDB015 (pMR-cfp-parB<sub>pr1</sub>) | Kan | s2 |
| GL506 | DH10B / pXDB025 (pKS-orfT-parS<sub>pr1</sub>) | Chlor | s2 |
| GL507 | DH10B / pXDB024 (pKS-orfT-parS<sub>pr1</sub>) | Chlor | s2 |
| GL511 | S17-1 λpir / pMQ414-romR (encodes RomR-tdTomato) | Gm | This study |
| GL573 | S17-1 λpir / pPROBE-NT | Kan | s3 |
| GL606 | NEB5α / pTNV215-tomato (PnptII-tomato-RSF1010-orfT-pT1A = pMQ414 without yeast maintenance sequences) | Gm | This study |
| GL611 | S17-1 λpir pTNV215-romR-tomato | Gm | This study |
| GL630 | S17-1 λpir / pK18mobsacB-Bd3895up-parS<sub>pm1</sub>-parB<sub>orT</sub>Bd3896down | Kan | This study |
| GL631 | S17-1 λpir / pK18mobsacB-Bd2052up-parS<sub>pr1</sub>-parB<sub>orT</sub>Bd2503down | Kan | This study |
| GL669 | TOP10 / pK18mobsacB | Kan | Lab collection |
| GL671 | S17-1 λpir / pK18mobsacB-dnaNup-dnaN-msfgfp-dnaNdow | Kan | This study |
| GL726 | MG1655 / pBAD18-msfgfp | Amp | This study |
| GL727 | MG1655 / pBAD18-mcherry | Amp | This study |
| GL728 | DH5α / pBG18 (pSEVA251-pBioFab-sfgfp) | Kan | Kind gift from C. Lesterlin (U. Lyon) |
| GL743 | S17-1 λpir / pTNV215-cfp-parB<sub>pr1</sub> | Gm | This study |
| GL744 | S17-1 λpir / pTNV215-ylp-parB<sub>PM1</sub> | Gm | This study |
| GL745 | S17-1 λpir / pTNV215-ylp-parB<sub>PM1</sub>-cfp-parB<sub>pr1</sub> | Gm | This study |
| GL809 | S17-1 λpir / pTNV215-cfp-parB<sub>pr1</sub>-romR-tomato | Gm | This study |
| GL831 | S17-1 λpir / pK18mobsacB-parB<sub>up</sub>-parB<sub>orT</sub>-msfgfp-parB<sub>udow</sub> | Kan | This study |
| GL832 | S17-1 λpir / pK18mobsacB-parB<sub>up</sub>-parB<sub>orT</sub>-mcherry-parB<sub>udow</sub> | Kan | This study |
| GL917 | S17-1 λpir / pTNV215-parB<sub>orT</sub>-mcherry | Gm | This study |
| GL918 | S17-1 λpir / pTNV215-parB<sub>orT</sub>-msfgfp | Gm | This study |
| GL972 | S17-1 λpir / pK18mobsacB-Bd0063-pBioFab-sfgfp-Bd0064 | Kan | This study |
| GL974 | S17-1 λpir / pK18mobsacB-Bd0063-pBioFab-sfTq2ox-Bd0064 | Kan | This study |
| GL975 | S17-1 λpir / pK18mobsacB-Bd0063-pBioFab-mcherry-Bd0064 | Kan | This study |
| GL985 | S17-1 λpir / pTNV215-parB<sub>orT</sub> | Gm | This study |
| GL1001 | S17-1 λpir / pTNV215-mcherry-parB<sub>PM1</sub> | Gm | This study |
| GL1223 | TOP10 / pTNV215-msfgfp | Gm | This study |
| GL1263 | S17-1 λpir / pTNV215-ylp-parB<sub>PM1</sub>-parB<sub>orT</sub>-mcherry | Gm | This study |
| GL1314 | S17-1 λpir / pK18mobsacB-dnaXup-dnaX-msfgfp-dnaXdow | Kan | This study |
| GL1420 | S17-1 λpir / pTNV215-PBd3471-cfp-parB<sub>pr1</sub> | Gm | This study |
| GL1424 | S17-1 λpir / pTNV215-PBd3471-ylp-parB<sub>pr1</sub> | Gm | This study |
| Strain     | Description                                                                 | Selectant | Source                                      |
|------------|------------------------------------------------------------------------------|-----------|---------------------------------------------|
| CJW6321    | MG1655 seqA::seqA-mcherry, constructed as CJW6324 in S4, here without the ftsZ-venus<sup>SW</sup> fusion | /         | Kind gift from C. Jacobs-Wagner (Stanford U.) |
| MT4401     | TOP10 / pMT679 (used to amplify mcherry)                                     | Chlor     | S5                                          |
|            | /                                                                            | Amp       | S6                                          |
|            | DH5α / pNM077 (used to amplify sfq2ox encoding monomeric superfolder Turquoise2ox) |           |                                             |
|            | /                                                                            | Amp       | S7                                          |
|            | TOP10 / pTNV162 (used to amplify mstgfp encoding monomeric superfolder GFP)   |           |                                             |
|            | /                                                                            | Amp       | Kind gift from L. Hamoen (U. Amsterdam)     |
|            | TOP10 / pTNV167 (used to amplify mstgfp encoding monomeric superfolder GFP)   |           |                                             |
|            | /                                                                            | Amp       | Kind gift from L. Hamoen (U. Amsterdam)     |
|            | TOP10 / pTNV143 (used to amplify mstgfp encoding monomeric superfolder GFP)   |           |                                             |

Table S1. Strain information: *E. coli* and *Bdellovibrio bacteriovorus* strains used in this study. Related to STAR Methods. *pBioFab is a constitutively active synthetic promoter; **PBd3471 is a *B. bacteriovorus* promoter active in attack phase."
| Plasmid description | Method of construction |
|--------------------|------------------------|
| pMQ414-romR, gm<sup>R</sup> | Assembly of *romR* (Bd2761) PCR-amplified from purified HD100 gDNA (oGL206/oGL207) with EcoRI-digested pMQ414 (GL495). |
| pTNV215-tdtomato, gm<sup>R</sup> | Assembly of PCR fragments of pMQ414 amplified with primers oGL283/oGL296, oGL281/oGL284, oGL280/oGL282, resulting in the removal of yeast maintenance elements from pMQ414 (GL495). |
| pTNV215-romR-tdtomato, gm<sup>R</sup> | Assembly of PCR fragments of pMQ414-romR amplified with primers oGL281/oGL283 and oGL280/oGL282, resulting in the removal of yeast maintenance elements from pMQ414-romR (GL511). |
| pK18mobsacB-Bd3895up-parS<sub>PMT1</sub>-Bd3896down, kan<sup>R</sup> | Assembly of the following DNA fragments: *Bd3895up* amplified from HD100 gDNA using primers oGL227/oGL228; *parS<sub>PMT1</sub>* amplified from plasmid in GL506 using primers oGL229/oGL230; *Bd3896down* amplified from HD100 gDNA using primers oGL231/oGL232; XbaI-digested pK18mobsacB (GL669). |
| pK18mobsacB-Bd2052up-parS<sub>P1</sub>-Bd2053down, kan<sup>R</sup> | Assembly of the following DNA fragments: *Bd2052up* amplified from HD100 gDNA using primers oGL233/oGL234; *parS<sub>P1</sub>* amplified from plasmid in GL507 using primers oGL235/oGL236; *Bd2053down* amplified from HD100 gDNA using primers oGL237/oGL238; XbaI-digested pK18mobsacB (GL669). |
| pK18mobsacB-dnaNup-msfgfp-dnaNdown, kan<sup>R</sup> | Assembly of the following PCR-amplified fragments: *Bd0002* amplified from HD100 gDNA using primers oGL395/oGL396; *msfgfp* amplified with oGL299/oGL300 from pTNV167 (GL597); *dnaNdown* amplified from HD100 gDNA using primers oGL397/oGL398; vector pK18mobsacB (GL669) amplified with primers oGL331/oGL332. |
| pBAD18-msfgfp, amp<sup>R</sup> | Assembly of PCR fragments amplified from pBAD18 with primers oGL321/oGL322, and from pTNV162 with primers oGL340/oGL326. |
| pBAD18-mcherry, amp<sup>R</sup> | Assembly of PCR fragments amplified from pBAD18 with primers oGL321/oGL322, and from pMT679 with primers oGL330/oGL509. |
| pTNV215-cfp-parB<sub>P1</sub>, gm<sup>R</sup> | Assembly of the following PCR-amplified fragments: *PnptII* amplified from pPROBE-NT vector (strain GL573) using primers oGL287/oGL288; *cpf-parB<sub>P1</sub>* amplified from plasmid in GL505 using primers oGL289/oGL559; pTNV215 vector amplified from pTNV215-tdtomato (GL606) using primers oGL451/oGL452. |
| pTNV215-yfp-parB<sub>PMT1</sub>, gm<sup>R</sup> | Assembly of the following PCR-amplified fragments: *PnptII* amplified from pPROBE-NT vector (strain GL573) using primers oGL287/oGL288; *yfp-parB<sub>PMT1</sub>* amplified from plasmid in GL504 using primers oGL291/oGL560; pTNV215 vector amplified from pTNV215-tdtomato (GL606) using primers oGL451/oGL452. |
| pTNV215-yfp-parB<sub>PMT1</sub>-cpf-parB<sub>P1</sub>, gm<sup>R</sup> | Assembly of the following PCR-amplified fragments: *PnptII* amplified from pPROBE-NT vector (strain GL573) using primers oGL287/oGL288; *yfp-parB<sub>PMT1</sub>* amplified from plasmid in GL504 using primers oGL291/oGL295; *cpf-parB<sub>P1</sub>* amplified from plasmid in GL505 using primers oGL293/oGL559; pTNV215 vector amplified from pTNV215-tdtomato (GL606) using primers oGL451/oGL452. |
| pTNV215-cfp-parB<sub>P1</sub>-romR-tdtomato, gm<sup>R</sup> | Assembly of the following PCR-amplified fragments: *romR-tdtomato* amplified from pTNV215-romR-tdtomato (GL611) using primers oGL702/oGL703; *cpf-parB<sub>P1</sub>* amplified from plasmid in GL743 using primers oGL700/oGL701; pTNV215 vector amplified from pTNV215-tdtomato (GL606) using primers oGL451/oGL452. |
| pK18mobsacB-parB<sub>romR</sub>-parB<sub>P1</sub>-mcherry-parB<sub>romR</sub>, kan<sup>R</sup> | Assembly of the following PCR-amplified fragments: *Bd3905up* amplified from HD100 gDNA of HD100 using primers oGL751/oGL752; *mcherry* amplified from plasmid in GL701 using primers oGL489/oGL209; *Bd3905down* amplified from HD100 gDNA using primers oGL755/oGL754; pK18mobsacB (GL669) amplified using primers oGL331/oGL332. |
| Plasmid Name | Construction Details |
|--------------|----------------------|
| pK18mobsacB-parB\textsubscript{up}\-parB\textsubscript{down}, kan\textsuperscript{R} | Assembly of the following PCR-amplified fragments: \textit{Bd}3905\textsubscript{up} amplified from HD100 gDNA using primers oGL751/oGL752; \textit{msfgfp} amplified using primers oGL299/oGL337 from pTNV167 (GL597); \textit{Bd}3905\textsubscript{down} amplified from HD100 gDNA using primers oGL753/oGL754; pK18mobsacB (GL669) amplified using primers oGL331/oGL332. |
| pTNV215-parB\textsubscript{sd}, gm\textsuperscript{R} | Assembly of \textit{parB\textsubscript{sd}}-mcherry amplified from plasmid in GL831 using primers oGL890/oGL891, and pTNV215 vector amplified from pTNV215-ttdtomato (GL606) using primers oGL451/oGL452. |
| pTNV215-parB\textsubscript{msfgfp}, gm\textsuperscript{R} | Assembly of \textit{parB\textsubscript{msfgfp}} amplified from plasmid in GL832 using primers oGL890/oGL892, and pTNV215 vector amplified from pTNV215-ttdtomato (GL606) using primers oGL451/oGL452. |
| pK18mobsacB-Bd\textsubscript{0063}\-pBioFab-sfgfp-Bd\textsubscript{0064}, kan\textsuperscript{R} | Assembly of the following PCR fragments: \textit{Bd}0063 amplified from HD100 gDNA using primers oGL961/oGL962; \textit{sfgfp} amplified with oGL963/oGL964 from pBG18 (GL728); \textit{Bd}0064 amplified from HD100 gDNA using primers oGL965/oGL966; vector pK18mobsacB (GL669) amplified with primers oGL264/oGL265. |
| pK18mobsacB-Bd\textsubscript{0063}\-pBioFab-sftq2ox-Bd\textsubscript{0064}, kan\textsuperscript{R} | Assembly of sftq2ox amplified from plasmid pNM077 (GL601) using primers oGL887/oGL898, and a vector fragment amplified from pK18mobsacB-Bd\textsubscript{0063}\-pBioFab-sfgfp-Bd\textsubscript{0064} (GL976) with primers oGL783/oGL967. |
| pK18mobsacB-Bd\textsubscript{0063}\-pBioFab-mcherry-Bd\textsubscript{0064}, kan\textsuperscript{R} | Assembly of \textit{mcherry} amplified from plasmid in GL832 using primers oGL887/oGL898, and a vector fragment amplified from pK18mobsacB-Bd\textsubscript{0063}\-pBioFab-sfgfp-Bd\textsubscript{0064} (GL976) with primers oGL783/oGL967. |
| pTNV215-parB\textsubscript{Bb}, gm\textsuperscript{R} | Assembly of \textit{parB\textsubscript{Bb}} amplified from HD100 gDNA using primers oGL890/oGL892, and pTNV215 vector amplified from pTNV215-ttdtomato (GL606) using primers oGL451/oGL452. |
| pTNV215-mcherry-parB\textsubscript{PnptII}, gm\textsuperscript{R} | Assembly of \textit{mcherry} amplified from plasmid in GL832 using primers oGL491/oGL338, and a fragment from plasmid in GL744 amplified using primers oGL452/oGL973. |
| pTNV215-msfgfp, gm\textsuperscript{R} | Assembly of PCR fragments amplified from pTNV215-ttdtomato (GL606) with primers oGL282/oGL362, and from pTNV143 (GL598) amplified with primers oGL363/oGL364. |
| pTNV215-yfp-parB\textsubscript{PnptII}\-parB\textsubscript{Bb}, mcherry, gm\textsuperscript{R} | Assembly of \textit{parB\textsubscript{Bb}}-mcherry amplified from plasmid in GL917 using primers oGL1117/oGL1118, and in plasmid in GL744 amplified using primers oGL1116/oGL451. |
| pK18mobsacB-dna\textsubscript{Xup}-dna\textsubscript{X-down}, kan\textsuperscript{R} | Assembly of the following PCR-amplified fragments: \textit{Bd}3731 amplified from HD100 gDNA using primers oGL520/oGL521; \textit{msfgfp} amplified with oGL299/oGL300 from pTNV167 (GL597); dna\textsubscript{X-down} amplified from HD100 gDNA using primers oGL522/oGL523; vector pK18mobsacB (GL669) amplified with primers oGL331/oGL332. |
| pTNV215-PBd3417-\textit{cfp}-parB\textsubscript{PnptII}, gm\textsuperscript{R} | Assembly of the following PCR-amplified fragments: \textit{PBd}3471 amplified from HD100 gDNA using primers oGL1302/oGL1303; pTNV215-\textit{cfp}-parB\textsubscript{PnptII} amplified with oGL1298/oGL1299, resulting in the replacement of the constitutively active PnptII promoter by the PBd3471 promoter. |
| pTNV215-PBd3417-yfp-\textit{parB\textsubscript{PnptII}}, gm\textsuperscript{R} | Assembly of the following PCR-amplified fragments: \textit{PBd}3471 amplified from HD100 gDNA using primers oGL1302/oGL1303; pTNV215-yfp-parB\textsubscript{PnptII} amplified with oGL1298/oGL1299, resulting in the replacement of the constitutively active PnptII promoter by the PBd3471 promoter. |

Table S2. Construction of plasmids used in this study. Related to STAR Methods.
Table S3. Construction of *Bdellovibrio bacteriovorus* strains used in this study. Related to STAR Methods.

| Strains | Construction method |
|---------|---------------------|
| GL512   | Mating GL499 x GL511 |
| GL673   | Mating GL499 x GL671, allelic replacement |
| GL676   | Mating GL734 x GL631, allelic replacement |
| GL771   | Mating GL676 x GL743 |
| GL772   | Mating GL676 x GL744 |
| GL773   | Mating GL676 x GL745 |
| GL784   | Mating GL734 x GL743 |
| GL785   | Mating GL734 x GL744 |
| GL806   | Mating GL734 x GL630, allelic replacement |
| GL816   | Mating GL676 x GL809 |
| GL867   | Mating GL806 x GL743 |
| GL868   | Mating GL806 x GL744 |
| GL869   | Mating GL806 x GL745 |
| GL870   | Mating GL806 x GL809 |
| GL906   | Mating GL734 x GL832, allelic replacement |
| GL909   | Mating GL806 x GL631, allelic replacement |
| GL995   | Mating GL909 x GL745 |
| GL1002  | Mating GL734 x GL917 |
| GL1003  | Mating GL734 x GL918 |
| GL1004  | Mating GL734 x GL919 |
| GL1023  | Mating GL734 x GL977, allelic replacement |
| GL1024  | Mating GL734 x GL978, allelic replacement |
| GL1025  | Mating GL734 x GL979, allelic replacement |
| GL1055  | Mating GL906 x GL671, allelic replacement |
| GL1102  | Mating GL806 x GL671, allelic replacement |
| GL1103  | Mating GL1102 x GL1001 |
| GL1121  | Mating GL906 x GL630, allelic replacement |
| GL1211  | Mating GL673 x GL612 |
| GL1212  | Mating GL734 x GL976, allelic replacement |
| GL1222  | Mating GL906 x GL631, allelic replacement |
| GL1261  | Mating GL734 x GL985 |
| GL1364  | Mating GL499 x GL1314, allelic replacement |
| GL1367  | Mating GL1121 x GL744 |
| GL1368  | Mating GL1122 x GL743 |
| GL1372  | Mating GL806 x GL1263 |
| GL1388  | Mating GL1025 x GL918 |
| GL1475  | Mating GL1420 x GL771 |
| GL1476  | Mating GL1424 x GL868 |
| Primer name | Primer sequence (5’>3’) |
|-------------|------------------------|
| oGL060      | cattcgccatcaggtgc      |
| oGL206      | tcaagacacagatgaagagAGTCCATATGGCTTTACGCGTCTTGC |
| oGL207      | tctgccctgtctcattcAGCTCAAGCTTGGTACATGCGACTTTTCAGTTTCCG |
| oGL209      | TAATTCCATATGGCTTTACGCGTCTTGC |
| oGL229      | gattatgtaaaAGCTTTCACACGCCAATTTCC |
| oGL230      | gcttccgattttGCCAGTTGAAAGCCGTTG |
| oGL231      | gttttcacaacctAAAGAAGGAGGCTTTG |
| oGL232      | aagcttgcatgctcaggtgatcAGCTAAGAGCTGCGGG |
| oGL233      | tcgaagcttccagctccggccactTTCAACTCTTTGGGATATGG |
| oGL234      | atggcgaagttttACTGGCTTTTTTGTTTATC |
| oGL235      | caaaaaagccagtAAACCTTTGCGCATTTCAATTTTC |
| oGL236      | tcgatccccggCCAGGTGAAATCGTGCC |
| oGL237      | gatttccacctggCCGCGGATCTGACTG |
| oGL238      | aagcttcagctctcagttgcagttgATGTTCCAGCGCGCAACTTC |
| oGL244      | atttgagcggataaca |
| oGL257      | cattgaccggatgagacttt |
| oGL258      | cttgcaaatattcgtgacct |
| oGL264      | TCTTAGCTGACCTGCGAG |
| oGL265      | GGATCCCCCGGTACGCGAG |
| oGL280      | cggccgataagtcctcaggtGTGATGAAAGGACC GGCTTTG |
| oGL281      | cctctcccggccgtttgcgccagttca |
| oGL282      | gaattctctcatctgtcttgatcata |
| oGL283      | gtgagctttactgccccagcctgca |
| oGL284      | gaccacccgcgtactgccggccagcca |
| oGL287      | cgactctagagatcccccgggtacCCCGGGACGCTGCGCGCAA |
| oGL288      | gaattctctcatctgtct |
| oGL289      | tcaagagacagatgagagagaaattcATGAGCTAAAAGGAAGAAGAATTTT |
| oGL291      | tcaagagacagatgagagaaattcATGCTAATGGTGAAGAAGAATTTT |
| oGL293      | ggaaagacttcagaggtaggtgagtt |
| oGL295      | cctgattcttagaagcttttcagag |
| oGL296      | gccggccgagtcagttcgctggt |
| oGL299      | ggtctcaggaagccggtcagATCCAAAGGA |
| oGL300      | ccgcgtactcgtccgccagcgGTAACGCGACCGGCCGTCAAGCTTTG |
| oGL304      | ggtgataacctggtgaaccgtat |
| oGL305      | ggtctgcgtttagtgcataag |
| oGL306      | gcgcgctggggagagcactcgaa |
| oGL316      | cagctcccgtcagaaagacagt |
| oGL331      | gcagctggccgctgttacca |
| oGL332      | gcggagctgggaagagcactcgaa |
| oGL338      | ggtcttcgagccgttcctcagagccCTTGTAACGCTGCTGCCATGCC |
| oGL343 | gcctctatgcctctctgacga |
|--------|------------------------|
| oGL344 | gcaaaaagcctgacgcttgga |
| oGL345 | gccccataacagcctgaacaaa |
| oGL346 | gcacaagggttcctgtatagact |
| oGL354 | gcaaccatttctgctcagtct |
| oGL355 | gcagacatttctcgaatagct |
| oGL371 | agacaggtaggaggaatttcatag |
| oGL381 | ccgcaacgacaagcccaactggca |
| oGL382 | ttctctgagcgaggactctgcatactatactgcccgctcactattat |
| oGL383 | gttgggctgcctttgtggttatttgtaactgtaatgtctgt |
| oGL387 | cgggtaccgaagctgctaatctg |
| oGL388 | ttgacagtctgcaccgggtgtactt |
| oGL389 | ttgagctcggtacccgggtgtactt |
| oGL390 | cgggtaccgagctgcagatttc |
| oGL391 | ttaacactgaagctgtatagct |
| oGL392 | cgggtaccgagctgcagatttc |
| oGL395 | acagctgcatctgattagct |
| oGL396 | gtgagctcagctgatccagtcgagcc |
| oGL397 | gctcggccgcttgctcagccc |
| oGL398 | gtaaaacgcggcagcagcagcag |
| oGL447 | atgtcgtgataattgctg |
| oGL448 | ttactgccacttctttta |
| oGL451 | ggtacgtcgtatagcttataata |
| oGL452 | gaattcttcctctgctcttgtatcag |
| oGL489 | ggapcggctcaggtgct |
| oGL491 | agacaggatgaggaatttcatag |
| oGL498 | gaagagcgcaccaatacagca |
| oGL520 | cggcctgactctcgcaccc |
| oGL521 | acagctgcatctgattagct |
| oGL522 | gttaaacagcgcggctgc |
| oGL523 | gctcggccgcttgctcagccc |
| oGL559 | ctgcgttctgatttacttctcagacttc |
| oGL560 | ctgcgttctgatttacttctcagacttc |
| oGL638 | gatgacgcagcacaatcat |
| oGL639 | cactctttcagaggtcag |
| oGL681 | tcaagagacaggagagaaaatttcagaggtaataggaag |
| oGL682 | tctgtatccagtcatctgagttcctcagct|
| oGL699 | gtaacggagtctgaccgggca |
| oGL700 | ctgtaaccgataaaagcggctgatcataaaacagcag |
| oGL701 | tgaattcttccttaagccggttactgtaatgtcaggg |
| oGL702 | ccgaagcccttaagaggaagatagtcgatitatagtact |
| oGL703 | tatacagacgcgttactcatgcggcagcga |
Table S4. Oligos used in this study. Related to STAR Methods. Overlapping sequences used for cloning by DNA assembly are highlighted in black.
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