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Chromosome organization by a conserved condensin-ParB system in the actinobacterium Corynebacterium glutamicum

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Higher-order chromosome folding and segregation are tightly regulated in all domains of life. In bacteria, details on nucleoid organization regulatory mechanisms and function remain poorly characterized, especially in non-model species. Here, we investigate the role of DNA-partitioning protein ParB and SMC condensin complexes in the actinobacterium Corynebacterium glutamicum. Chromosome conformation capture reveals SMC-mediated long-range interactions around ten centromere-like parS sites clustered at the replication origin (oriC). At least one oriC-proximal parS site is necessary for reliable chromosome segregation. We use chromatin immunoprecipitation and photoactivated single-molecule localization microscopy to show the formation of distinct, parS-dependent ParB-nucleoprotein subclusters. We further show that SMC/ScpAB complexes, loaded via ParB at parS sites, mediate chromosomal inter-arm contacts (as previously shown in Bacillus subtilis). However, the MukBEF-like SMC complex MksBEFG does not contribute to chromosomal DNA-folding; instead, this complex is involved in plasmid maintenance and interacts with the polar oriC-tethering factor DivIVA. Our results complement current models of ParB-SMC/ScpAB crosstalk and show that some condensin complexes evolved functions that are apparently uncoupled from chromosome folding.

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Ech organism must complete genome replication and separation in the course of one cell cycle prior to cell division in concert with transcriptional processes. To this end, chromosomes are highly organized structures in terms of segregation and overall folding patterns. The functional organization of bacterial genomes, structured into the nucleoid, has been predominantly investigated in a limited number of model species, e.g., *Escherichia coli*, *Vibrio cholerae*, *Bacillus subtilis*, or *Caulobacter crescentus*, revealing diverse levels of compaction and segregation strategies.

ParABS systems and condensins are two (nearly) ubiquitous bacterial enzyme machineries that contribute to chromosome homeostasis. With a few exceptions among γ-proteobacteria, including *E. coli*, all branches of bacteria and several Archaea harbor *parS* sites that recruit partitioning protein ParB. The ParABS system contains one or several *parS* sites usually in the vicinity to the chromosomal origin of replication (*oriC*). ParB proteins bind to these sequence-specific motives and form large nucleocomplexes by spreading and three-dimensional (3D)-bridging between ParB dimers, resulting in large chromosome interaction domains promoting encompassing the *oriC*, which have been revealed by chromosome conformation capture coupled to deep sequencing (Hi-C) for *B. subtilis*. In an alternative model termed nucleation and caging, ParB nucleation at *parS* is stabilized by dynamic ParB dimer–dimer interactions and weak interactions with nonspecific DNA generating a scaffold for locally high ParB concentrations confined around *parS*. The ParB segregation is driven by a ParA ATPase, which binds nonspecifically to the nucleoid and is released from DNA upon ATP hydrolysis triggered by transient ParB interactions. In the course of chromosome replication, ParB-orig complexes act in combination with ParA as Brownian ratchets along dynamic DNA loci: slow ParA-DNA rebinding rates generate ParA gradients, which serve as tracks for directed movement of partition complexes away from their sisters. Perturbation of the system by placing *parS* sites at ectopic, *oriC*-distal regions can cause severe DNA-segregation phenotypes. To date, only a few studies investigated the impact of chromosomal *parS* localization on DNA segregation and folding.

In addition to ParABS systems, most bacteria harbor condensin complexes, members of the structural maintenance of chromosome (SMCs) family of proteins found in all kingdoms of life. In standard model organisms, condensins are equally essential for faithful chromosome segregation by compacting DNA into separate nucleoids. The SMC/ScpAB complex is well-studied in *B. subtilis*, where it consists of two large SMC subunits and the kleisin ScpA associated with dimeric accessory protein ScpB that assemble into a ring-like structure. A recent study suggests progressive extrusion of condensin-encircled DNA loops upon conformational changes in the SMC subunit, which leads to a gradual size increase of trapped DNA molecules. The active process(es) driving DNA extrusion allow(s) for translocation along the chromosome with velocities of around 50 kb/min (ref. 31) and depend(s) on the ATPase activity of SMC. To be loaded on *parS* sites, SMC/ScpAB complexes necessitate ParB. They redistribute to the same replichores from stochastically positioned chromosomal arms, where it consists of two large SMC subunits and the kleisin ScpA associated with dimeric accessory protein ScpB that assemble into a ring-like structure. All other *parS* sites were predicted earlier in *Corynebacterium glutamicum*. A BLAST analysis pointed at ten *B. subtilis*-like 16 bp consensus sequences in *C. glutamicum*, localized in one cluster within a 35 Kb region at 73 Kb from *oriC* (1% of the 3.21 Mb chromosome; Fig. 1a). Out of the ten *parS* sites, only the furthest from *oriC* (*parS1*) lies within a coding sequence (trpCF). All other *parS* sequences (labeled *parS* 2-10) were positioned within intergenic regions. Degenerated *parS* sequences with at least three base-pair mismatches were also identified further away from *oriC*, e.g., 5′ of cg0146 or within the *fusA* and *cg1994* coding region. To test whether these putative *parS* were responsible for the recruitment of ParB, chromatin immunoprecipitation (ChIP) of ParB was performed with a strain harboring a mCherry-tagged version of the native ParB (note that all mutant strains used in this study derive from clean allelic replacements and have, unless otherwise noted, a wild-type-like phenotype). Distinct and very reproducible enrichment signals were obtained at the ten *parS* sites close to *oriC* (*parS1*-10 at 3.16 MB) (Fig. 1a), whereas the degenerated *parS* sequences failed to recruit ParB. Additional smaller peaks were identified at highly transcribed DNA regions, in particular at ribosomal genes, transfer RNA gene clusters, and at all of the ribosomal RNA operons (Fig. 1a). Magnification of the *oriC* region reveals three distinct ParB propagation zones overlapping with *parS1*-4, *parS5*-8, and *parS9*-10, respectively (Fig. 1b). Remarkably, those three regions seem to recruit these species all contain a single condensin complex, yet a broad range of bacteria possesses combinations of SMC/ScpAB and MksBEFG (MukB-like SMC), for which functional characterizations are non-existent to date. Current work in bacteria and in eukaryotes convey the general assumption that all SMCs are likely to play role(s) in chromosome organization. In bacteria, it remains unknown why some species harbor more than one type of condensin, and whether and how they would work in concert with each other and coordinate with systems such as ParABS.

In this work, we used a combination of high-resolution microscopy and genomic chromosome conformation capture (3C/Hi-C) to unveil the global organization of the diploid *Corynebacterium glutamicum* genome. *C. glutamicum* is a polar growing actinobacterium, whose genome encodes both SMC/ScpAB and MksBEFG. In this species, the two *oriC* are continuously associated with the polar scaffold protein DivIVA, whereas newly replicated sister *oriC* segregate towards division septa via the ParABS system. In contrast to *B. subtilis*, *C. glutamicum* ParAB are by themselves crucially important drivers of reliable nucleoid separation prior to cell division, where ParAB deletions yield in 20% of anucleate daughter cells. Here, analyses of chromosomal ParB-binding patterns evince ten redundant *parS* sites, which mediate ParB subcluster formation at *oriC*. A single *parS* site maintains ParB propagation over 32 kb neighboring regions and is sufficient to promote the SMC-dependent alignment of the two chromosomal arms. Hi-C also reveals SMC-dependent long-range contacts surrounding *oriC*. In contrast to SMC, we show that the polar positioned MksBEFG condensin acts mostly on plasmid transmission to daughter cells, without obvious influence on nucleoid architecture.

### Results

**Chromosome segregation is governed by ten *oriC*-proximal *parS* sites.** Previous studies on *C. glutamicum* chromosome partitioning have revealed two stable ParB-orig complexes at each cell pole, whereas newly replicated origins are segregated towards a division septum formed at midcell. In *B. subtilis*, *C. crescentus* and *Pseudomonas aeruginosa* ParAB-mediated chromosome segregation and folding depends on *parS* sites. A single *parS* site maintains ParB propagation over 32 kb neighboring regions and is sufficient to promote the SMC-dependent alignment of the two chromosomal arms. Hi-C also reveals SMC-dependent long-range contacts surrounding *oriC*. In contrast to SMC, we show that the polar positioned MksBEFG condensin acts mostly on plasmid transmission to daughter cells, without obvious influence on nucleoid architecture.
decreasing amounts of ParB, from parS1-4 (most enriched) to parS9-10 (less enriched). As all parS sites are identical in sequence, differences in ParB recruitment might result from the number and distance of parS sequences in the context of the overall nucleoid folding patterns at the oriC region.

**Higher-order organization of the C. glutamicum chromosome.**

In *B. subtilis*, SMC-mediated chromosome folding initiates at ParB-parS clusters surrounding the oriC, bridging the two repli-chores with each other. To characterize whether *C. glutamicum* parS sites play a similar role in the overall organization of the chromosome, we applied a Hi-C-like approach to exponentially growing wild-type cells (Methods). The genome-wide contact map, displaying the average contact frequencies between all 5 Kb segments of wild-type chromosomes (Fig. 1c) displayed the following 3D features. First, a strong and broad diagonal reflecting frequent local contacts between adjacent loci and observed in all Hi-C experiments. Second, chromatin interaction domains (CIDs), i.e., self-interacting regions previously described in *C. crescentus* and other species (Fig. 1c and Supplementary Fig. 1) (11 domains detected at a 200 Kb resolution). In *C. glutamicum*, 6 out of 11 boundaries are associated with high transcriptional activity or gene lengths (Supplementary Fig. 1A). The Hi-C signal did not present overall a clear correlation with transcriptional activity (Supplementary Fig. 1B). Other roadblocks like nucleoid-associated proteins might play a major role in confining chromosomal interaction domains. Third, a secondary diagonal perpendicular to the main one and extending from the ori-proximal, 35 Kb parS cluster (Fig. 1d, white dashed line) down to the replication terminus. This structure shows that the two repli-chores are bridged over their entire length, as in *B. subtilis* (Fig. 1d, black triangle on the sides of the contact matrix), as in *B. subtilis* (Fig. 1c, dark triangle on the contact matrix).
might be due to the segregation and translocation of the ParB-oriC complex along the nucleoid during segregation when oriCs reposition at midcell. This signal is also maximal at the parS cluster and not at oriC locus (Fig. 1c, d). This observation reinforces the fact that the parS cluster is at the tip of Corynebacterium chromosome fold and is one of the main actors of chromosome segregation. A similarity matrix between the different constructed Hi-C matrices was calculated (Supplementary Fig. 2A) and flow cytometry was performed with all samples used for Hi-C analyses to control for chromosome number and potential replication differences (Supplementary Fig. 2B–D).

A single parS site is sufficient to maintain chromosome architecture. As all parS sites are in close proximity on the C. glutamicum chromosome, we tested the importance of ParB-parS complex titration for the overall chromosome organization. Cells with chromosomes carrying the single parS1 site (for parS mutations, see Supplementary Fig. 3A) grow and divide like wild-type cells (Supplementary Figs. 2A and 3B). However, the removal of all ten parS sites resulted in a cell length phenotype (Supplementary Fig. 3B) and 29% DNA-free mini-cells, hinting to a nucleoid segregation defect similar to the ΔparB phenotype (Fig. 2a and Supplementary Table 1). We further analyzed ParB localization in mutant strains carrying either a single or no parS site. First, if only parS1 is present, cellular localization of fluorescent ParB-mCherry foci is similar to wild type, positioning at cell poles and migrating to the newly formed septa11 (Fig. 2b). Interestingly, the combination of a single parS site with ParB-eYFP resulted in 7% anucleate mini-cells (Supplementary Fig. 3C and Supplementary Table 1), reflecting functional constraints of the ParB-eYFP fusion in the presence of only one parS site. Therefore, the high number of chromosomal parS sites likely evolved to improve the robustness of the segregation machinery. ParB ChIP-quantitative PCR (qPCR) signals of locus parS1 were similar in both wild-type and mutant strains (Fig. 2c). ParB spreading around the single parS site was characterized through ChIP-seq analysis (Fig. 2d and Supplementary Fig. 4), where ParB binding was maximum within 2 Kb windows on both sides of parS, while extending up to 16 Kb on either side. However, redundancy of parS sites is not restricted to parS1 in C. glutamicum, as exemplified by the analysis of the single parS10, which was equally sufficient for wild-type-like growth and morphology (Supplementary Fig. 3D–E). A single parS10 site recruits ParB exclusively within the third nucleation zone encompassing 26 Kb (Supplementary Fig. 3A, F). We next investigated the role of parS sites and ParB in the overall chromosome folding by performing Hi-C in mutants (Fig. 2e, f). The absence of either ParB or of all parS sites led to the disappearance of the secondary diagonal. In addition, the cross-shaped pattern reflecting contacts between the ori and the whole chromosome disappears in those mutants, also illustrated by the ratio between wild-type and mutant contact maps (Fig. 2f). This result shows that parS sites and ParB are two major structural components of chromosome organization and act in the same pathway to recruit downstream factors that fold the chromosome emanating from the parS cluster, and bridge the two chromosomal arms together down to the replication terminus region. The contact map of the strain deleted for parS2-10, but carrying a single parS1, maintains a secondary diagonal, showing that parS1 alone is sufficient to ensure the loading of ParB and the overall folding of the chromosome (Fig. 2e, f). However, some differences appeared between the wild type and the single parS1 site contact maps. In the mutant, the large domain surrounding the oriC shows minor differences in the contact maps compared with wild type, suggesting that a single parS site is not sufficient to fully restore the complexity of Corynebacterium chromosome ori folding (Fig. 2e, f).

The single parS site was then repositioned at different genomic regions. Cells harboring an ectopic, single parS site at 9.5°, 90°, 180°, or 270° positions were viable (Supplementary Fig. 5A–C). Unlike cells harboring parS1 at its original position, ParB-parS complexes distribute virtually randomly along the longitudinal cell axis in all of these mutants (Supplementary Fig. 5C), resulting in ~25% anucleate cells (Supplementary Table 1). Therefore, parS shifts result in nucleoid segregation defects. The number of ParB foci nevertheless correlates well with cell length (Supplementary Fig. 5D), excluding replication initiation deficiencies. ParB binding to a parS sequence positioned at the 90° chromosomal position (locus cg0904, strain CBK042) was identified in a 9 Kb range on either side of parS (Supplementary Figs. 4A, 5E), approximately half the ParB-propagation distance determined for cells harboring one parS at its native locus. We also analyzed the mutant harboring parS at 90° chromosomal position by Hi-C (CBK037). The contact map of this mutant displays a "bow shape" or a hairpin motif at the position of the aberrant parS sequence (Supplementary Fig. 6), reminiscent to the one observed in E. coli at the level of the oriC-distal parS site and pointing a local folding of the chromosome (Supplementary Fig. 6A). Collectively, these results show a redundancy of parS sites, with an optimal function confined to the oriC-proximal region.

Palm identifies ParB subclusters. To directly characterize oriC domain compaction via ParB, we applied photoactivated localization microscopy (PALM) to visualize individual ParB-PAmCherry molecules with nanometer resolution (~20 nm localization precision). PALM revealed distinct ParB-dense regions at cell poles and quarter position regions, similar to foci observed via diffraction-limited epifluorescence microscopy (Fig. 2g). These ParB-enriched regions (macroclusters) display heterogeneous densities, with a variable number of higher density zones within subclusters. Macro- and subclusters have been identified via the OPTICS algorithm that orders data points according to their spatially closest neighbors for identification of clustering structures49,50 (see Methods and Supplementary Fig. 7A) and analyzed in strains harboring a single, two, or all the parS sites (Fig. 2g and Supplementary Fig. 7B). We define a macrocluster as 32 individual events being localized within a maximum distance of 50 nm for macroclusters and 35 nm for subclusters, yielding in cluster numbers that are in line with ParB epifluorescence data and oriC numbers determined by flow cytometry (Supplementary Table 2). It is noteworthy that high chromosome numbers promote inter-molecular oriC colocalization in fast-growing cells. For more accurate cluster estimations, PALM analysis was performed using slow-growing cells resulting in significantly fewer ParB macroclusters per cell (Supplementary Fig. 7C)41. As segregation of oriC complexes might alter their DNA compaction, we focused on the two largest macroclusters per cell, stably tethered at cell poles. Although this is not a direct measurement of the number of ParB nucleation points (parS), a strain with a higher number of parS sites can be expected to result in higher ParB density variability when compared with one which contain a single or no nucleation point. The amount of ParB contained within each macrocluster in wild type is significantly higher than in cells containing the single parS1 site (Fig. 2h), in agreement with the ParB deposition observed via ChIP-seq. A parallel between PALM and ChIP-seq can also be drawn with respect to the number of subclusters per macrocluster, with a higher number of subclusters in the wild type that accordingly harbors three ParB nucleation zones along the parS cluster compared with the single parS site forming only one zone (Fig. 2h). Absence of all parS sites likewise results in a significant
reduction of ParB macrocluster size and subcluster numbers compared with wild type (Supplementary Fig. 7D). These differences were not observed when comparing cells harboring all or two parS sites (parS1,10), which harbors two distinct ParB nucleation regions surrounding parS1 and parS10 (Supplementary Figs. 4A, 5F, and 7E). These observations could explain the differences observed between contact matrices of wild type and ΔparS 2–10 strains, and the higher structuring of the oriC domain when only one parS1 site is present. We therefore conclude that the architecture of the C. glutamicum partition complex is dependent on parS, and that ParB-parS nucleoprotein complexes are visible as individual subclusters.
**Fig. 2** A single parS site mediates chromosome folding. a One parS site is necessary and sufficient for wild type-like morphology and nucleoid segregation. Phase-contrast images of exponentially grown chromosome harboring either all (WT), one (parS2-10mut, CBK023), or none (parS1-10mut, CBK024) parS site(s), or lacking parB (ΔparB, CDC003) are shown. DNA is stained with Hoechst (yellow). Scale bar, 2 µm. b Fluorescence microscopy analysis of parB::ParB-mCherry (shown in green) in wild type (CBK006), parS2-10mut (CBK027), and parS1-10mut backgrounds (CBK028). Absence of parS leads to diffuse cellular ParB localizations. Scale bar, 2 µm. c ChIP-qPCR for strains described before, normalized to wild-type parS1 signal (mean ± SD, n = 3). d ChIP-seq of C. glutamicum parB::ParB-mCherry parS2-10mut (black) at a 3.1-3.2 Mb chromosomal range. Wild-type-like propagation (green) of ParB protein around parS1-4; 0.5 Kb bin size. Location of parS site positions in wild type or mutant sequences are indicated (gray lines). e Normalized contact maps of ΔparB, parS1-10mut, and parS2-10mut mutants centered at oriC (CDC003, CBK024, and CBK023). Color codes as in Fig. 1. f Differential maps correspond to the log2 of the ratio (wild-type norm/mutant norm); color scales indicate contact enrichment in mutant (blue) or wild type (red) (white indicates no differences between the two conditions). g Single molecule localization microscopy analysis of parS2-10mut (CBK009 and CBK029). Top: Gaussian rendering of parB::ParB-mCherry signals (0.71 PSF, 1 px = 10 nm), below: color-coded representation of_parB::ParB-mCherry events within corresponding cells49; all events (light blue), macromolecular assemblies (dark blue) and subclusters (yellow) are indicated. Scale bar, 0.5 µm. See Methods and Supplementary Fig. 7 for details. h Comparison of ParB::ParB-mCherry cluster properties. Only the two biggest clusters per cell were taken into account for analyses; significant differences between conditions are indicated by small letters above all bars. Left: events per macromolecule, medians are indicated as solid lines, and whiskers mark 1.5 IQRs (interquartile ranges); clusters; wild type: n = 130; clusters; parS2-10mut: n = 143. Right: subcluster numbers per macromolecule shown as overlay bar chart for both strains. Number of subcluster per macromolecule (two-tailed Kruskal-Wallis rank-sum test: $\chi^2$ = 228.4, df = 1, $p = 0.0004569$) and macromolecule size (two-tailed Kruskal-Wallis rank-sum test: $\chi^2$ = 27.582, df = 1, $p = 1.506e-07$) differ significantly between source. Data are provided as a Source Data file.

**C. glutamicum harbors two paralogous condensin complexes.** In bacteria, the condensin paralog complexes SMC/ScpAB and, in *E. coli* and *B. subtilis*, MksBEF, are key factors of chromosome folding10,21,36,38. MksBEF (for MukBEF-like SMC) is another condensin occasionally found in bacterial genomes40, whose role(s) remain(s) obscure. A sequence homology search of the C. glutamicum genome pointed at the presence of both SMC/ScpAB and MksBEF. The SMC/kleisin is encoded by genes *smc* and *mksGBEF* (Fig. 3a), whereas the Mks complex is encoded on a widely conserved operon40 and comprises genes *mksG*: *mksE*: and *mksF*: *mksG*: (Fig. 3a), including MksG, which was being suggested to interact in complex with MksBEF40.

To characterize condensin complex formation in vivo, mass spectrometry of anti-mCherry pulldown experiments using SMC-mCherry and MksB-mCherry as baits of whole-cell lysates were performed. Wild-type-like growth of corresponding strains and stability of SMC and MksB fluorescent fusions were confirmed (Supplementary Fig. 8A, C). Kleisin subunit ScpA and ScpB co-precipitated significantly with SMC compared with the negative control containing free mCherry, whereas subunits MksF and MksE, but not MksG, were substantially enriched in the MksB pulldown experiments (Supplementary Fig. 8D). ParB, which fluorescence-tagged version of core subunit SMC was imaged, yielding a minor fraction of anucleate cells (4–5%) (Supplementary Fig. 9A, C and Supplementary Table 1), indicating that SMC and ParB function in the same pathway and with ParB being epistatic to SMC. Hence, a functional interaction of SMC and ParB proteins regulating chromosome organization is likely. To further determine cellular localization of SMC/ScpAB complexes, a strain harboring a fluorescently tagged version of core subunit SMC was imaged, revealing the formation of SMC clusters along the entire longitudinal axis of the cell (Fig. 3d). Clusters of SMC and ParB investigated in a strain carrying both labeled complexes (parB::ParB-mNeonGreen *smc*: *smc*-mCherry) are often proximal but do not always colocalize, whereas the foci numbers correlate with cell length (Fig. 3e). Up to eight SMC-mCherry foci were counted per cell. On average, cells contained fewer SMC-foci than ParB nucleoprotein complexes (Supplementary Fig. 8B). To further characterize the role of SMC, we generated Hi-C contact maps of the mutant (Fig. 3f). Deletion of *smc* abolishes the secondary diagonal in the maps (Fig. 3f), indicating that SMC and ParB function in the same pathway and have a synthetic phenotype concerning the cohesion of the two chromosomal arms. The combination of *smc* and *parB* mutations mimics a *parB* phenotype (Supplementary Fig. 9A, D), again resulting in the loss of contacts between chromosomal arms and further in the loss of the segregation signal described before (Fig. 3f, g). However, ΔparB and Δsmc contact maps display different patterns along the main diagonal, suggesting that those two proteins affect differently chromosome architecture of *C. glutamicum* (Supplementary Fig. 9E). Therefore, it appears that an interplay of SMC/ScpAB with ParB is responsible for replichore cohesion in *C. glutamicum*, similar to *B. subtilis* and *C. crescentus* each harboring only one type of condensin complex10,21,22,36.

**SMC-mediated cohesion of chromosomal arms.** We aimed to characterize *C. glutamicum* condensin SMC/ScpAB. Mutation of the SMC/ScpAB complex causes a conditionally lethal phenotype due to chromosome mis-segregation in *B. subtilis*25. In sharp contrast, a *smc* deletion in *C. glutamicum* did not result in either growth defects, DNA-segregation defects, or aberrant cell length distributions and morphologies compared with the wild type in minimal or complex media (Supplementary Fig. 9A, B and Supplementary Table 1). Nonetheless, the combination of genetic backgrounds *parB*: *parB*-eYFP and Δsmc yield a minor fraction of anucleate cells (4–5%) (Supplementary Fig. 9A, C and Supplementary Table 1), indicating that SMC and ParB function in the same pathway and with ParB being epistatic to SMC. Hence, a functional interaction of SMC and ParB proteins regulating chromosome organization is likely. To further determine cellular localization of SMC/ScpAB complexes, a strain harboring a fluorescently tagged version of core subunit SMC was imaged, revealing the formation of SMC clusters along the entire longitudinal axis of the cell (Fig. 3d). Clusters of SMC and ParB investigated in a strain carrying both labeled complexes (parB::ParB-mNeonGreen *smc*: *smc*-mCherry) are often proximal but do not always colocalize, whereas the foci numbers correlate with cell length (Fig. 3e). Up to eight SMC-mCherry foci were counted per cell. On average, cells contained fewer SMC-foci than ParB nucleoprotein complexes (Supplementary Fig. 8B). To further characterize the role of SMC, we generated Hi-C contact maps of the mutant (Fig. 3f). Deletion of *smc* abolishes the secondary diagonal in the maps (Fig. 3f), indicating that SMC and ParB function in the same pathway and have a synthetic phenotype concerning the cohesion of the two chromosomal arms. The combination of *smc* and *parB* mutations mimics a *parB* phenotype (Supplementary Fig. 9A, D), again resulting in the loss of contacts between chromosomal arms and further in the loss of the segregation signal described before (Fig. 3f, g). However, ΔparB and Δsmc contact maps display different patterns along the main diagonal, suggesting that those two proteins affect differently chromosome architecture of *C. glutamicum* (Supplementary Fig. 9E). Therefore, it appears that an interplay of SMC/ScpAB with ParB is responsible for replichore cohesion in *C. glutamicum*, similar to *B. subtilis* and *C. crescentus* each harboring only one type of condensin complex10,21,22,36.
mCherry foci are less frequent in the absence of ParB or parS (Supplementary Fig. 10C). These findings suggest that ParB promote condensin loading onto DNA at oriC-proximal parS sites. In addition, ChIP-seq revealed that SMC concentrates at a 13 Kb region upstream parS1 (Fig. 4a). SMC enrichment in this region was lost following a partial deletion of this locus and its reinsertion at another genomic position (Supplementary Fig. 10D-F) or following its substitution by a random DNA sequence (Supplementary Fig. 10D, G). Therefore, the accumulation of SMC at the 13 Kb region in the vicinity of parS sites points at roadblocks that trap SMC rather than specific SMC binding. This hypothesis is further supported by the study of the
Fig. 3 Functional characterization of two SMC-like complexes in *C. glutamicum*. a Sections of the *C. glutamicum* genome map indicating localizations of condensin subunit genes. b Confirmation of protein–protein interactions via bacterial two-hybrid screen. Interactions were quantified by β-galactosidase assays in all combinations of hybrid proteins: ParB<sup>ΔΔ</sup>; ParB mutant R175A (mean ± SD, n = 3). c Illustration of SMC/ScpAB and MksBEFG subunit interactions based on bacterial two-hybrid data; cartoons indicate condensin complex formations. d Top: dependence of ParB foci numbers on cell length in *C. glutamicum* wild type (WT) and Δ<sup>smc</sup>Δ<sup>mksB</sup> (ΔΔ, CBK011) cells grown in BHI (n > 350). Linear regression lines are shown r(WT) = 0.57, r(ΔΔ) = 0.62; slopes and intercepts are equal (ANOVA, F(1, 770) = 0.059, p = 0.808; ANCOVA, F(1, 771) = 0.60, p = 0.4391). Below: cellular localization of condensin subunits in *C. glutamicum* Δ<sup>smc</sup>∷<sup>smc-mCherry</sup> and Δ<sup>mksB</sup>∷<sup>mksB-mCherry</sup> cells (CBK012, CBK015). Microscopy images exemplify cellular mCherry fluorescence of SMC (left) and MksB (right); white lines indicate cell outlines. Scale bar, 2 µm. e Top: SMC and ParB foci numbers positively correlate with cell length in double labeled strain Δ<sup>parB</sup>∷<sup>parB-mNeonGreen</sup> (CBK013), r(ParB) = 0.74, r(SMC) = 0.53 (n > 350). Below: subcellular localization of ParB and SMC is exemplified in representative cells shown in overlays between mNeonGreen and mCherry fluorescence, and in separate channels. Scale bar, 2 µm. f Normalized contact maps of Δ<sup>smc</sup>, Δ<sup>mksB</sup>, Δ<sup>parB</sup>/Δ<sup>smc</sup>, and Δ<sup>smc</sup>/Δ<sup>mksB</sup> mutants (CDC026, CBK001, CBK002, and CBK004), displayed as in Fig. 1. Source data are provided as a Source Data file.

Fig. 4 Chromosomal SMC loading is mediated by ParB at parS sites. a SMC enrichment at parS sites (gray) is ParB-dependent. ChIP-seq of ParB-mCherry (green; CBK006 and CBK047) and SMC-mCherry (orange; CBK012, CBK014, CBK051, and CBK049) in strain backgrounds as indicated. Depicted are chromosomal ranges of 3.1–3.2 Mb, bin size 0.5 Kb. b Whole-genome ChIP-seq data of strains harboring SMC-mCherry wild type (gray, CBK012) or E1084Q mutant (orange, CBK050). SMC enrichment at parS sites and at other loci (red letters), in particular tRNA gene clusters and at rRNA genes (a–f) is illustrated in 0.5 Kb bins in linear scale along the chromosome with an x-axis centered at oriC. c Normalized contact map of mutant strains parB∷<sup>R175A</sup> (CBK047) and d the corresponding differential map indicating the log of the ratio (wild-type norm/mutant norm) as in Fig. 2.
contact map of wild type cells (Fig. 1c, d and Supplementary Figs. 1 and 11). Indeed, the SMC enrichment region is clearly delimited by a strong border on its left (Supplementary Fig. 1, Directional Index at 100 Kb resolution and Supplementary Fig. 11, red dashed line). In the absence of ParB or SMC (Supplementary Fig. 11), the strong border observed in Hi-C maps is shifted towards parS sites. Therefore, this border originates from a combination of multiple processes.

SMC is also recruited to parS inserted in ectopic positions, e.g., the 90° parS-insertion (Supplementary Figs. 5, 6). Indeed, in the absence of SMC (Supplementary Fig. 6), the bow-shape motif is no longer present at the ectopic parS site, demonstrating that chromosomal arm cohesion is SMC-dependent, and that artificial loading of SMC at non-native positions is not sufficient to fold the entire chromosome. We further assayed chromosomal SMC-loading sites by making use of a well-characterized SMC ATP-hydrolysis mutant E1084Q32,53. SMCΔfl−mids pBHK18 and pWK0 were enriched 60- and 10-fold compared with wild-type ParB fluorescence intensities were low compared with DivIVA. The relative localization of MksB and DivIVA has also been observed via PALM microscopy. Here we can see that the MksB foci composed of the highest number of localizations typically localize at the poles and are surrounded by DivIVA itself (Supplementary Fig. 13B). Although no quantitative analysis has been performed, the number of visible foci in the imaged cells does not differ with what has been already observed via conventional fluorescence microscopy (Fig. 5a). Moreover, we applied Hi-C to characterize the role of MksB in genome folding in the different mutants (Fig. 31). In contrast to smc, deletion of mksB had no effect at large scale on chromosome organization, as shown by the ratio map between the wild type and the mutant (Fig. 3 g). Moreover, Δsmc and ΔsmcΔmksB contact maps were nearly identical (Fig. 31), showing that MksB and SMC are most likely not involved in the same process(es). Finally, we applied the software HiCrep on our various Hi-C map, a framework for assessing the reproducibility of Hi-C data56 (Supplementary Fig. 2A). Strain backgrounds ΔparB, ΔparS, and ΔparBΔsmc Hi-C maps appear to form a first cluster; Δsmc and ΔsmcΔmksB appear to form a second cluster; finally, wild type, ΔparSΔ2-10, and ΔmksB form a third cluster. This result strongly suggests that MksB does not significantly affect chromosome architecture in C. glutamicum. ChIP-seq of MksB failed to detect specific loading sites along the C. glutamicum chromosome (Fig. 5d), supporting the hypothesis that MksB, unlike other bacterial condensins studied so far, plays no direct or indirect role in C. glutamicum chromosome organization. Therefore, we analyzed its impact on the maintenance of extrachromosomal DNA. The MksBEGF complex appears involved in plasmid maintenance, as shown by the qPCR copy number analysis of two low-copy number (pBHK18 and pWK0) and two high-copy number (pJCI and pEKO) E. coli– C. glutamicum shuttle vectors sized 3.5–6 Kb. High-copy number plasmids derive from cryptic C. glutamicum plasmids57,58, whereas replicons of both low-copy number plasmids originate from a plasmid isolated from the closely related Corynebacterium diphtheriae59,60. In ΔmksB mutants, both low-copy number plasmids pBHK18 and pWK0 were enriched 60- and 10-fold compared with wild type, when grown in the absence of selection marker (Fig. 5e). On the contrary, the amount of high-copy number vectors per cell was hardly affected. A Δsmc control did not result in a significant increase of plasmid levels compared with wild type (Fig. 5e). We confirmed these findings by plasmid extractions from C. glutamicum cells lacking MksB that yielded exceptionally large quantities of pBHK18 and pWK0, turning them into high-copy number plasmids under these conditions (Fig. 5f). By contrast, amounts of pJCI and pEKO did not differ notably compared with control strains. These analyses show a MksB-dependent decrease in plasmid level, specifically of the ΔparB phenotype, excluding redundancy of condensin functions in chromosome segregation (Supplementary Fig. 9D). Further, oriC-ParB foci numbers (Fig. 3d) and their spatiotemporal localization (Supplementary Fig. 9A) remain largely unaffected upon deletion of smc and mksB. MksB fluorescence was mainly detected at the cell poles (Fig. 3d), further supporting an interaction with the polar protein DivIVA. To test cellular MksB-DivIVA colocalization in more detail, we constructed a dual-reporter strain harboring MksB-mCherry in combination with DivIVA-mNeonGreen, which grows and divides in comparison with the wild type (Fig. 5a, Supplementary Fig. 13A, and Supplementary Table 1). Individual protein fluorescence patterns of MksB and DivIVA are displayed in large-scale demograph analyses (Fig. 5b). Averaged fluorescence profiles along longitudinal cell axes extracted from still microscopy images show colocalization of MksB and DivIVA at cell poles and division septa prior to cytokinesis in long cells (Fig. 5b, c) even if cellular MksB fluorescence intensities are low compared with DivIVA. The relative localization of MksB and DivIVA has also been observed via PALM microscopy. We can see that the MksB foci composed of the highest number of localizations typically localize at the poles and are surrounded by DivIVA itself (Supplementary Fig. 13B). Although no quantitative analysis has been performed, the number of visible foci in the imaged cells does not differ with what has been already observed via conventional fluorescence microscopy (Fig. 5a). Moreover, we applied Hi-C to characterize the role of MksB in genome folding in the different mutants (Fig. 31). In contrast to smc, deletion of mksB had no effect at large scale on chromosome organization, as shown by the ratio map between the wild type and the mutant (Fig. 3 g). Moreover, Δsmc and ΔsmcΔmksB contact maps were nearly identical (Fig. 31), showing that MksB and SMC are most likely not involved in the same process(es). Finally, we applied the software HiCrep on our various Hi-C map, a framework for assessing the reproducibility of Hi-C data56 (Supplementary Fig. 2A). Strain backgrounds ΔparB, ΔparS, and ΔparBΔsmc Hi-C maps appear to form a first cluster; Δsmc and ΔsmcΔmksB appear to form a second cluster; finally, wild type, ΔparSΔ2-10, and ΔmksB form a third cluster. This result strongly suggests that MksB does not significantly affect chromosome architecture in C. glutamicum. ChIP-seq of MksB failed to detect specific loading sites along the C. glutamicum chromosome (Fig. 5d), supporting the hypothesis that MksB, unlike other bacterial condensins studied so far, plays no direct or indirect role in C. glutamicum chromosome organization. Therefore, we analyzed its impact on the maintenance of extrachromosomal DNA. 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We confirmed these findings by plasmid extractions from C. glutamicum cells lacking MksB that yielded exceptionally large quantities of pBHK18 and pWK0, turning them into high-copy number plasmids under these conditions (Fig. 5f). By contrast, amounts of pJCI and pEKO did not differ notably compared with control strains. These analyses show a MksB-dependent decrease in plasmid level, specifically of
low-copy number plasmids. Notably, we observed emerging susceptibility of cells towards the pBHK18 selection antibiotic in the absence of MksB when testing its stability in plating assays and, therefore, cannot exclude side effects of MksB on the expression of antibiotic resistance. Subcellular MksB-mCherry localization was further assessed in the absence and presence of pEK0 or pWK0. The presence of plasmids does not have an impact on cell growth and morphology (Supplementary Fig. 13C) and wild-type-like MksB foci numbers per cell were detected (Supplementary Fig. 13D). Celluar MksB fluorescence profiles were further extracted (Supplementary Fig. 13E) and sorted by cell length in demographs showing MksB localization at cell poles and frequently at midcell prior to cytokinesis for all conditions (Supplementary Fig. 13E, F). However, polar MksB fluorescence tends to be more defined in cell populations harboring plasmids (Supplementary Fig. 13E, F).
Altogether, our data show that the two condensins in *C. glutamicum* evolved very different functions: whereas SMC/ScpAB act with ParB to promote replicon pairing and origin domain organization, MksBEFG does not organize chromosome architecture and seems involved in plasmid maintenance through a mechanism that remains to be characterized.

**Discussion**

Condensins are widely conserved enzyme machineries, which have been implicated in chromosome organization of pro- and eukaryotes. For long, it was considered that bacterial genomes encode one condensin complex that would either be of the Smc/ScpAB type as found in *B. subtilis* and *C. crescentus* or the MukBEF complex encoded in *E. coli* and related proteobacteria. However, recent reports suggested the existence of two or even multiple condensin systems in a single species. Yet, the underlying mechanisms and the precise function of these two condensin systems remained largely untested. We report here that the Gram-positive actinobacterium *C. glutamicum* also contains SMC/ScpAB and the Muk-like MksBEFG complexes. We set out to address the individual functions of the two condensin systems. Surprisingly our data provide clear evidence that the class of MksBEFG proteins do not work as chromosomal interactors; thus, the function of bacterial condensins in promoting DNA segregation to daughter cells is not generally conserved. A recent bioinformatics study predicted a role for MksBEFG complexes (termed Wadjet system) in plasmid-related defense, where homologous complex expression conveyed protection against the uptake of a high-copy number plasmid. However, function of MksBEFG in its native host had not been addressed before. We could show that the Mks system is indeed involved in the control of plasmid copy numbers, and that there is no involvement of this system in chromosome organization. As low-copy number vectors used in this study harbor replicons of a related *Corynebacterium* genus, the impact of MksBEFG on plasmids may be based on adaptations of plasmid-specific characteristics to the host species, such as the structural organization of the replication origin or replication-associated proteins. Our findings share a fascinating similarity to specific eukaryotic condensing homologs such as Rad50, being the closest eukaryotic relative to MukB/MksB. It was recently shown that Rad50–CARD9 complexes sense foreign cytoplasmic DNA in mammalian cells acting in innate immune responses against viral DNA. In addition, the more distantly related eukaryotic SMC5/6 complex had been shown to act in a defense mechanism against circular hepatitis B virus DNA, resembling the specific effect of prokaryotic MksBEFG on plasmid. Together, our data lend support to the notion that condensins’ function in innate immunity is an ancient mechanism. However, notably, we provide evidence that the MksBEFG complex is the only known condensin amongst pro- and eukaryotes to date that exclusively impacts on non-chromosomal DNA. For MksBEFG systems, it has been proposed that a fourth subunit, MksG, is important for function in plasmid maintenance. We could verify that MksG is part of the MksBEF complex of *C. glutamicum*. This assumption is in line with divergent functions observed between *C. glutamicum* MksBEFG and the structurally related *P. aeruginosa* MksBEF complex that is assumed to act in chromosome organization due to a synthetic DNA-segregation phenotype in combination with SMC/ScpAB. The direct interaction of a Mks complex with a polar scaffold protein such as the *C. glutamicum* DivIVA has not been described before. A challenging question for the future will be to determine the detailed mechanism of MksBEFG in plasmid defense and the putative role of the DivIVA–Mks interaction in this process.

We further describe here that SMC/ScpAB is indeed the major factor of replicon cohesion and chromosome organization in *C. glutamicum*. Like in *B. subtilis*, SMC is preferentially loaded onto the chromosome by a ParB/parS loading complex before spreading to the entire chromosome. The mild DNA-partitioning defects of a *smc* deletion in combination with a ParB-eYFP modification (Supplementary Table 1) strongly suggest a supportive role of SMC/ScpAB in the process of nucleoid separation, yet the *smc* phenotype appears to be entirely compensated by ParB. Therefore, our data demonstrate that the conserved role for SMC in chromosome organization and maintenance is also maintained in *C. glutamicum*. Moreover, bacterial two-hybrid analyses of SMC/ScpAB subunits evidence a self-interaction of *C. glutamicum* kleisin ScpA (Fig. 3), which has not been described in other organisms before. Based on this result, we speculate that *C. glutamicum* SMC/ScpAB might form dimers via kleisin subunits similar to *E. coli* MukBEF complex. These data point to a handcuffing model, where two SMC/ScpAB complexes are physically coupled together and translocate in pairs along the chromosome, similar as suggested for *B. subtilis*. We further describe a new phenotype for a ParB, point mutation in *C. glutamicum* that decreases SMC recruitment or blocks SMC release from its loading site. Building on this, we observe a weak interaction signal of ParB in bacterial two-hybrid analyses. Alternatively, SMC/ScpAB remains indirectly entrapped in higher-order ParB nucleiocomplexes, which possess altered DNA-folding properties. In either case, this mutant underlines the crosstalk between SMC/ScpAB and ParB nucleoprotein complexes in bacterial nucleoid organization.

Analysis of ParB complexes using two-dimensional (2D) PALM reveals ParB-dense regions within clusters that correlate to the number of ParB-enrichment zones along adjacent parS sites. In line with a current study on ParB cluster-assembly in *V. cholerae*, we suggest that these subclusters derive from independent nucleation and caging events, which merge into one ParB-macrocomplex per oriC in *C. glutamicum*. Presence of a single parS site leads to formation of almost globular ParB densities. Using Hi-C approaches, we further show that parS sites and ParB are major factors of chromosome folding in *C. glutamicum* as previously shown in other organisms. *C. glutamicum* chromosome adopts a global folding with a strong cohesion between the two chromosomal arms as expected from a bacterium harboring a longitudinal chromosomal organization similar to *B. subtilis* and, to a lesser extent, *C. crescentus*. Our analysis also suggests the existence of a chromosomal domain at parS sites in *C. glutamicum* as previously observed in *B. subtilis*, but with important differences: parS sites in *C. glutamicum* are only found on one side of the oriC locus and appeared to be at the edge of the nucleoid structure as observed in *C. crescentus*. A hairpin structure as it was observed in *B. subtilis* is absent in *C. glutamicum*. Contact maps of a strain with an ectopic parS site feature a bow-shaped structure reflecting an asymmetry in arm interaction, which has been shown before in *B. subtilis* and *C. crescentus*. Zipping of the chromosome is not complete and the ectopic parS site does not reorient the entire chromosome. Therefore, additional factors are involved in chromosome localization that supplement polar ParB-parS binding to DivIVA.

Importantly, we describe ParB-parS-dependent DNA contacts of the parS region with the entire nucleoid, indicating that oriC segregation occurs across the entire nucleoid. This is in accord with the ori-ter configuration of the nucleoid in *C. glutamicum*. Different from *C. glutamicum*, *B. subtilis* SMC is required for segregation signals that do not spread along the whole chromosomal length. Based on our data, we propose the following model shown in Supplementary Fig. 14: organisms with polarly localized oriCs and a longitudinal chromosome organization rely...
on ParAB for oriC segregation, as they can use the DNA scaffold as a track. By contrast, species with a central replication factory cannot efficiently use ParAB. B. subtilis is an exception, since here a longitudinal chromosome orientation is present during sporulation and, hence, ParAB (spor/syj) phenotypes are only obvious during spore formation. Segregation of otherwise transversally arranged B. subtilis chromosomes during vegetative growth rely on an initial SMC-driven segregation along a limited fraction of the nucleoid instead. Consequently, SMC/ScpAB-mediated replicon coherence is likely dispensable for oriC segregation in bacteria with a strict longitudinal chromosome arrangement that allows for efficient ParABS-driven chromosome partitioning.

Methods

Bacterial strains, plasmids, and oligonucleotides. Primers, plasmids, and strains used in this study are listed in Supplementary Data 1 and 2.

For protein–protein interaction screens, genes of interest were amplified via PCR, digested with respective enzymes, and ligated into bacterial two-hybrid vectors. E. coli DH5α were utilized for plasmid cloning. Genes DivIVA and parB/parR1775A were amplified using primer pairs DivIVA-XbaI-F/DivIVA-BamHI-R and ParB-XbaI-F/ParB-BamHI-R from genomic DNA or pK19mobsacB-parBR175A, and resulting fragments were digested with XbaI/BamHI. For amplification of scpB, scpA, mksE, mksF, and mksG, primer pairs ScpA-XbaI-F/ScpB-XbaI-R, ScpA-BamHI-F/MksE-BamHI-R, MksF-BamHI-F/MksG-XbaI-R, MksF- XbaI-F/MksG-XmaI-R, and MksG*-XbaI-F/MksG*-XmaI-R were utilized, followed by restriction digests with XbaI/XmaI. Primer pairs SMC-XbaI-F/SMC-KpnI-R and MksB*-XbaI-F/MksB*-KpnI-R were used for PCR amplification of genes scpB and mksB, which were subsequently digested with XbaI/KpnI or XbaI/KpnI. To increase the distance of XmaI and KpnI restriction sites, a short sequence was inserted in between these sites by overhanging PCRs using primers pUT18c-mcs-HindIII-F, pUT18c-mcs-Puvel-F, pKNT25-mcs-Nhel-F, or pKNT25-mcs-HindIII-F in combination with pUT18c-P(K)N25-kns-F/R for plasmids pUT18c, pUT18c, pKNT25, and pKNT25, respectively. Resulting fragments and corresponding vectors were digested with HindIII and KpnI and subsequently ligated, resulting in plasmids pUT18s, pUT18c, pKNT25, and pKNT25, respectively. All digested gene fragments mentioned above were digested into pUT18, pUT18c, pKNT25, or pKNT25 or pUT18mcs, pUT18c-mcs, pKNT25-mcs, and pKNT25-mcs, respectively.

Derivatives of the suicide integration vector pK19mobsacB were used for clean allelic replacements in C. glutamicum, containing the modified genomic region of interest including its 500 bp up- and downstream homologous flanking sequences. Plasmid cloning was performed using E. coli DH5α.

To construct pK19mobsacB Δsmc 500 bp upstream and downstream of smc were PCR amplified using primer pairs Δsmc-BamHI-F/Δsmc-SphI-R and Δsmc-BamHI-F/Δsmc-EcoRI-R, respectively. Both fragments served as templates in an overhanging PCR, yielding a 1000 bp fragment, which was digested with BamHI and EcoRI and subsequently ligated into pK19mobsacB. pK19mobsacB Δsmc was constructed accordingly, using primer pairs Δsmc-BamHI-F/Δsmc-SphI-R and Δsmc-BamHI-F/Δsmc-EcoRI-R as PCR primers. Each fragment pair served as template in an overhang PCR, yielding a 1000 bp fragment, which was digested with BamHI and EcoRI, and fragments were digested with HindIII/SalI, XmaI/EcoRI, or SalI/HindIII. Genomic integration of pK19mobsacB plasmids were selected on kanamycin, or SacI restriction site 3°′ and E1084Q-D-F/E1084Q-BamHI-D-R, which further yield in an E1084Q mutation and an additional XbaI restriction site 3°′.

As p55 and parS, as well as parS9 and parS10, are localized in close proximity on the genome (<100 bp distance), their deletions were accomplished using in each case one plasmid for both parS sites. For construction of pK19mobsacB parS5 5°′-mut, 5°′ genomic region upstream of parS5, downstream of parS5, and in between, both were PCR amplified using primer pairs ParB-N-ter-SalI-F/ParB-N-ter-BamHI-R and ParB-C-ter-SalI-F/ParB-C-ter-BamHI-R, respectively. Each fragment pair served as template in an overhanging PCR, yielding a 1000 bp fragment, which was digested with BamHI and EcoRI, and fragments were digested with HindIII/SalI, XmaI/EcoRI, or SalI/HindIII in combination with SalI and HindIII for restriction digestion. For construction of pK19mobsacB ΔmksB up- and downstream regions of mksB were PCR amplified using primers AmkaΔB-HindIII-up-F/AmkaΔB-PstI-up-R and AmkaΔB-PstI-D/F/AmkaΔB-XbaI-D-R. Resulting 500 bp fragments were digested with HindIII/ PstI and PstI/XbaI and consecutively ligated into pK19mobsacB.

Fluorescent C-terminal fusions of ParB protein with PAmCherry or mNeonGreen were obtained by utilizing plasmids pK19mobsacB-paRB-mNeonGreen and pK19mobsacB-paRB-PAmCherry. To this end, the yEPF sequence of plasmid pK19mobsacB-paRB-yEPF10 was replaced by respective fluorescent sequence coding plasmid pK19mobsacB-paRB-pAmCherry or pK19mobsacB-paRB-mNeonGreen. To construct pK19mobsacB-mNeonGreen, 5°′ genomic region of interest including its 500 bp up- and downstream homologous flanking sequences. Plasmid cloning was performed using E. coli DH5α.

To construct pK19mobsacB Δsmc 500 bp upstream and downstream of smc were PCR amplified using primer pairs Δsmc-BamHI-F/Δsmc-SphI-R and Δsmc-BamHI-F/Δsmc-EcoRI-R, respectively. Both fragments served as templates in an overhanging PCR, yielding a 1000 bp fragment, which was digested with BamHI and EcoRI and subsequently ligated into pK19mobsacB. pK19mobsacB Δsmc was constructed accordingly, using primer pairs Δsmc-BamHI-F/Δsmc-SphI-R and Δsmc-BamHI-F/Δsmc-EcoRI-R as PCR primers. Each fragment pair served as template in an overhang PCR, yielding a 1000 bp fragment, which was digested with BamHI and EcoRI, and fragments were digested with HindIII/SalI, XmaI/EcoRI, or SalI/HindIII in combination with SalI and HindIII for restriction digestion. For construction of pK19mobsacB ΔmksB up- and downstream regions of mksB were PCR amplified using primers AmkaΔB-HindIII-up-F/AmkaΔB-PstI-up-R and AmkaΔB-PstI-D/F/AmkaΔB-XbaI-D-R. Resulting 500 bp fragments were digested with HindIII/ PstI and PstI/XbaI and consecutively ligated into pK19mobsacB.
Screening of allelic replacements in C. glutamicum using primer pairs. 

Plasmid extraction from subsequent construction of strains CBK012 and CBK015, further including strain CBK052 as negative control. Lysate of exponentially grown cells was used for immunoprecipitation via magnetic RFP-Trap® agarose beads. For proteomic analysis samples were further processed and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify and quantify proteins in all samples.

To verify the occurrence of allelic replacements, strains CBK012, CBK015, and CBK052 were cultured in H medium using culture flags pretreated with 0.5% sodium hypochlorite. CBK052 was induced at OD600 ~1 with 0.5 mM isopropyl β-1-thiogalactopyranoside (IPTG). Exponentially growing cells (OD600 = 3, 10 mL) were collected once in 10 mL washing buffer (10 mM HCl, pH 7.5 and 10 mM NaCl; 150 mM EDTA 0.5 mM) and resuspended in 1.5 mL washing buffer supplemented with 1 mM phenylmethylsulfonyl fluoride in EtOH. All following steps were performed at 4 °C. After cell disruption via FastPrep® (MP Biomedicals) at 10 × 6.5 m/s, 30 s cell debris was removed by centrifugation at 18,000 × g. Immunoprecipitation was performed with 25 μl magnetic RFP-Trap® agarose beads (Chromotek) incubated in 1 mL lysate for 1 h. Thereafter, beads were washed three times in washing buffer and again washed three times in 100 mM ammonium bicarbonate prior to storage at −20 °C.

For proteomic analysis of interacting proteins, the magnetic beads were first washed with 50 μl of 100 mM TRIS pH 7.6. Subsequently, 50 μl of 100 mM TRIS pH 7.6 containing 4 μm urea, 5 μm dithiothreitol for reduction of disulfide bond, and 0.2 μg of LysC for predigestion of proteins were added to each sample. After incubation of 3 h, 100 μl of 100 mM TRIS pH 7.6 and 10 mM iodoacetamide were added for blocking of free cysteine side chains and samples were incubated in the dark for 5 min. Samples were diluted with 100 μl TRIS pH 7.6 to reduce the urea concentration and 1 μg of trypsin was added to each sample. The samples were incubated for 14 h to complete protein digestion and subsequently trifluoroacetic acid was added to a final concentration of 0.5% to acidify the samples. Peptide mixture was separated from the magnetic beads before the desalting step. The beads were washed 2× with 75 μl of 0.1% formic acid (FA) and the wash solvent was aspirated. For sample desalting, peptides were stamped from C18 discs (Empore C18, 3 M) and placed into a 200 μl pipette tip. Following binding of peptides, stage tips were washed 2× with 60 μl of 0.1% FA and peptides were eluted with 40 μl acetonitrile containing 30% methanol and 0.1% FA. Samples were dried in a speedvac for 5 min. For proteomic analysis of all samples, samples were analyzed by LC-MS/MS to identify and quantify proteins in all samples. First, peptides were separated by nano-reversed phase chromatography using a linear gradient from 2 to 35% acetonitrile over 50 min in 0.1% FA on an in-house-packed chromatography column in a nano-electrospray emitter tip. Eluting peptides were directly infused into the mass spectrometer (QExactive, Thermo Fisher) and detected in positive ionization mode. The operating cycle was programmed to detect peptides in the range from 300 to 1600 m/z and up to 10 precursors were selected for MSMS analysis by CID fragmentation. Precursor ions required a charge state between +2 and +6 and a minimal signal intensity of 6 × 104.

Protein mapping and quantitative analysis raw LC-MS/MS data were searched against a C. glutamicum database retrieved from Uniprot (vs. 03/2017, 3093 protein entries) using a forward/reversed search by the Andromeda algorithm within the MaxQuant software suite. Peptide hits were searched with 17 p.p.m. precursor mass deviation in the first search and 3 p.p.m. for the main search. For MS/MS, the tolerance was 25 p.p.m. A mass accuracy of 200 ppm (50 ppm tolerance, 25 p.p.m. for the main search). Carbamidomethylation of cysteine was the only fixed modification. Peptide match results were sorted by their probability score and filtered for 2% reversed peptide hits and 5% reversed protein hits. To calculate protein enrichments and significance values, reversed protein hits and proteins with less than three quantitative values in any of the three sample types (control, mksB IP, and smc IP) were filtered out. The iBAQ-values were log2 transformed and median normalized. In case of one missing value in the triplicate measurements, the value was imputed using a closest neighbor method; for more missing data points, a random value from a standard distribution downweighted by a factor of 1.8 from the sample distribution and width of 0.3 was selected. Samples were compared using a Student’s t-test, which was false discovery rate controlled by sample permutation.

Bacterial two-hybrid screening. Protein interactions obtained by mass spectrometry were confirmed via bacterial two-hybrid assays, using compatible vectors expressing adenylate cyclase subunits T25 and T18 (pKt25/ pKt25T and pUT18/ pUT18C). E. coli BHT101 co-transformed with respective vectors were plated on indicator medium LB/X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 40 μg/mL), supplemented with 50 μg/mL kanamycin and pKt25T or pUT18C. After incubation of plates overnight for color development, OD of growth was adjusted to an OΔ600 of 0.5 for BHI and to an OΔ600 of 1 for growth in CGXII medium. Kanamycin (25 μg/mL) was added where applicable.

Protein identification via immunoprecipitation and mass spectrometry. Immunoprecipitation of SMc and MksB interaction partners was performed with magnetic RFP-Trap® agarose beads and analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify and quantify proteins in all samples. Following IP steps, immunoprecipitations, proteins, strains CBK012, CBK015, and CBK052 were cultured in BHI medium using culture flags pretreated with 0.5% sodium hypochlorite. CBK052 was induced at OD600 ~1 with 0.5 mM isopropyl β-1-thiogalactopyranoside (IPTG). Exponentially growing cells (OD600 = 3, 10 mL) were collected once in 10 mL washing buffer (10 mM HCl, pH 7.5 and 10 mM NaCl; 150 mM EDTA 0.5 mM) and resuspended in 1.5 mL washing buffer supplemented with 1 mM phenylmethylsulfonyl fluoride in EtOH. All following steps were performed at 4 °C. After cell disruption via FastPrep® (MP Biomedicals) at 10 × 6.5 m/s, 30 s cell debris was removed by centrifugation at 18,000 × g. Immunoprecipitation was performed with 25 μl magnetic RFP-Trap® agarose beads (Chromotek) incubated in 1 mL lysate for 1 h. Thereafter, beads were washed three times in washing buffer and again washed three times in 100 mM ammonium bicarbonate prior to storage at −20 °C.

For proteomic analysis of interacting proteins, the magnetic beads were first washed with 50 μl of 100 mM TRIS pH 7.6. Subsequently, 50 μl of 100 mM TRIS pH 7.6 containing 4 μm urea, 5 μm dithiothreitol for reduction of disulfide bond, and 0.2 μg of LysC for predigestion of proteins were added to each sample. After incubation of 3 h, 100 μl of 100 mM TRIS pH 7.6 and 10 mM iodoacetamide were added for blocking of free cysteine side chains and samples were incubated in the dark for 5 min. Samples were diluted with 100 μl TRIS pH 7.6 to reduce the urea concentration and 1 μg of trypsin was added to each sample. The samples were incubated for 14 h to complete protein digestion and subsequently trifluoroacetic acid was added to a final concentration of 0.5% to acidify the samples. Peptide mixture was separated from the magnetic beads before the desalting step. The beads were washed 2× with 75 μl of 0.1% formic acid (FA) and the wash solvent was aspirated. For sample desalting, peptides were stamped from C18 discs (Empore C18, 3 M) and placed into a 200 μl pipette tip. Following binding of peptides, stage tips were washed 2× with 60 μl of 0.1% FA and peptides were eluted with 40 μl acetonitrile containing 30% methanol and 0.1% FA. Samples were dried in a speedvac for 5 min. For proteomic analysis of all samples, samples were analyzed by LC-MS/MS to identify and quantify proteins in all samples. First, peptides were separated by nano-reversed phase chromatography using a linear gradient from 2 to 35% acetonitrile over 50 min in 0.1% FA on an in-house-packed chromatography column in a nano-electrospray emitter tip. Eluting peptides were directly infused into the mass spectrometer (QExactive, Thermo Fisher) and detected in positive ionization mode. The operating cycle was programmed to detect peptides in the range from 300 to 1600 m/z and up to 10 precursors were selected for MSMS analysis by CID fragmentation. Precursor ions required a charge state between +2 and +6 and a minimal signal intensity of 6 × 104.
After centrifugation at 1000 × g for 10 min, clear lysates were transferred to a clean 96-well plate and 20 μl of 2-Nitrophenyl β-D-galactopyranoside (4 mg/ml in 50 mM Tris-HCl containing 0.1% Triton X-100, pH 7.4) was added at 30°C. The absorbance activity was recorded until the addition of 30 μl of Na₂CO₃ (1 M). Absorbance was determined at OD420 using a Tecan plate reader. Co-transformants harboring empty plasmids or pUT18C-zip/pKT25-zip plasmids served as positive and negative controls. Miller units of negative controls served as reference and were set to zero. Miller units of any other samples were normalized accordingly. All C- and N-terminal combinations of hybrid proteins were assayed and positive signals were confirmed through at least three replicates.

Fluorescence microscopy. Fluorescence microscopy was performed with exponentially grown cells mounted on agarose coated slides (1% agarose). Images were acquired on an Axio-Imager M1 fluorescence microscope (Carl Zeiss) with an EC Plan Neofluar ×100/1.3 oil Ph3 objective and a 2.5x optovar. Fluorescence of protein fusions with GFP (enhanced yellow, EYFP), YFP, mCherry (mCherry2), and mCherry/mCherry2 or DNA stained via Hoechst 33342 (1 μg/ml; Thermo Scientific) were determined using filter sets 46 HE YFP (EX BP 500/25, BS FT 515, and EM BP 535/30), 43 HE Cy 3 shift free (EX BP 550/25, BS FT 570, and EM BP 605/70), and 49 DAPI shift free (EX G 365, BS FT 395, and EM BP 445/50). Live-cell imaging and detection of photobleaching occurred with PrOn (1% in RH and loaded in a microfluidic chamber (B04A CellASIC®, Onix); the environmental chamber was heated to 30°C and 0.75 psi was applied for nutrient supply throughout the experiment. Images were taken in 5 min intervals. For display of cellular fluorescence profiles sorted by cell length, FIJI and R software were utilized57,75,79.

ChIP combined with sequencing. Briefly, cells were crosslinked (1% formaldehyde) for 30 min at room temperature (RT) and lysed. DNA was sheared by sonication, incubated with a m-Cherry antibody for 2 h at 4°C, and subsequently washed at 4°C. DNA purification was followed by library preparation and sequencing using an Illumina MiSeq system. Reads were aligned to the C. glutamicum ATCC 13002 genome sequence (GenBankID: BX971471.1). Further data analysis was performed using online tools77. More in details, reads were aligned with BWA, sorted with SAM tools (SAMtools), and the blat program was used to find alignments with m-Cherry-tagged versions. Exponentially growing cells were crosslinked in 1% formaldehyde for 30 min at RT; for SMC- and MskB-mCherry ChIP experiments, cells were treated with Crosslink Gold (Diagenode) for 30 min at RT and washed twice in phosphate-buffered saline (PBS) and finally diluted in buffer containing 10 mM glycine, cells were sedimented at 5000 × g for 10 min, finally resuspended in TES buffer (10 mM Tris-HCl pH 8, 10 mM EDTA, and 1% SDS). Protein G (Thermo Fisher Scientific) was bound to an a-m-Cherry Funtibody (BioVision, Inc.) in buffer L for 1 h at 4°C. After washing in buffer L, and subsequently incubated with cell extract for 2 h at 4°C. Thereafter, beads were washed in buffer L, in buffer L (50 mM HEPEs-KOH pH 7.5, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate), in buffer W (10 mM Tris-HCl pH 8, 250 mM LiCl, 0.5% NP-40, 0.01% deoxycholate, 1 mM EDTA), and TE buffer (10 mM Tris-HCl pH 8, 1 mM EDTA) consecutively and finally resuspended in TES buffer (10 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS). Extract samples were also supplemented with TES buffer and SDS to a final concentration of 1% SDS and corresponding ChIP sample was each normalized based on read counts and the ratio of the number of peaks per 0.5 kb bin were determined via the Galaxy web platform77,78.

Real-time PCR. DNA amplification was performed using a 2× qPCR Mastermix (KAPA SYBR®FAST, Peqlab) according to the manufacturer’s instruction, where reaction volumes of 10 μl contained 200 nM oligonucleotides and 4 μl of diluted DNA, respectively. Samples were measured in technical duplicates via an iQ5 Real-Time PCR detection system. The real-time PCR amplification was performed using the Bio-Rad IQ5 software version 2.1. Primer efficiencies were estimated by calibration dilution curves and slope calculation79; data were analyzed via the 2−ΔΔCt method accounting for dilution factors and sample volumes used for DNA purification. qPCR data of ChIP samples were normalized according to the previously described MksB@mCherry2 signal obtained at locus parS1 in the wild-type background, serving as reference in each experiment.

Protein purification. ParB protein production was performed in E. coli BL21 pLySs via the pET-16b vector-based system. Cells were grown in LB at 37°C; gene expression was induced adding 1 mM IPTG following growth for 12 h at 18°C. Subsequently, cells were suspended in washing buffer (50 mM Tris-HCl pH 7.4; 100 mM NaCl, 5 mM; MgCl₂, 1 mM dithiothreitol) containing EDTA-free proteinase inhibitor cocktail (Complete®, Roche) and DNaseI, and lysed using a high pressure cell homogenizer. Cell debris and membranes were removed by centrifugation at 4°C, 1700 g for 20 min, and 150,000 × g for 45 min, respectively. Thereupon, batch purifications of His-tagged protein were performed under native conditions using Ni-NTA agarose (ProteinA®, Machery-Nagel) according to manufacturer’s instruction. In brief, the equilibrated gel was incubated with clarified lysate for 60 min at 4°C under gentle agitation and washed twice in washing buffer containing 80 mM imidazole. Proteins were eluted in three steps using washing buffer with an imidazole concentration of 300 mM, concentrated via Amicon filter units (Merck) and further purified by applying size exclusion chromatography using an AKTA purifier system with a Superdex™ 200 gel filtration column (GE Healthcare Life Sciences).

Electrophoretic mobility shift assay. DNA-ParB binding was assayed using purified protein and double-stranded DNA fragments of ~1100 bp length with or without two parS sites. Fragments were generated by PCRs of a C. glutamicum genomic locus surrounding parS9 and parS10 using primer pairs parS9-mutin-HindIII up/ParS10mut-ECori-D-R. ParB concentrations of 0.05–25 μg were incubated with 3 ng DNA for 30 min at 30°C following sample separation in native gels (3–12% polyacrylamide, ServaGel™). DNA was stained using SYBR® Green I (Invitrogen).

Photolocalized activation microscopy. C. glutamicum cells were fixed with 3% formaldehyde prior to super-resolution imaging using a Zeiss ELYRA P.1 microscope with laser lines HR diode 305 nm and HR DPPS 200 mW 561 nm, and an Andor EMCCD iXon DU 897 camera. Cellular ParB-PamCherry@ChIP complexes were further analyzed using a photolocalized activation microscopy as described below. A small fraction of distinct protein clusters was carried out by applying the OPTICS algorithm in R80,81. For sample preparation, C. glutamicum cells expressing ParB-PamCherry@ChIP were collected in exponential growth phases, washed twice in PBS, and fixated in PBS + 3% formaldehyde solution (36.5–38.5% in H₂O + 0.1–10% methanol, Sigma Aldrich) for at least 30 min. Excess formaldehyde was subsequently quenched by adding 10 mM glycine, cells were sedimented at 5000 x g for 1 min, resuspended in PBS containing 10 mM glycine, and incubated for 5 min at RT. This quenching step was repeated three times; cells were finally diluted in buffer containing 50 mM Tris pH 7.4, 50 mM NaCl, 10 mM EDTA, and 0.5 μM sucrose (TSEMS). Cells expressing MksB-PamCherry with C. glutivA-mNeonGreen were not fixed due to the low amount of MksB expressed (formaldehyde fixation renders part of the fluorophore population unable to fluoresce) but simply collected and washed in TSEMS buffer.

Super-resolution imaging was performed on a Zeiss ELYRA P.1 microscope (laser lines HR diode 305 nm and HR DPPS 200 mW 561 nm). Cellular PamCherry-tagged proteins were detected via an Andor EMCCD iXon DU 897 camera as described before, using a long-pass 570 nm filter (LP570) and an alpha Plan-Apochromat ×100/1.46 Oil DIC M27 objective for imaging. Further, 100 nm TetraSpeck microspheres and the implemented drift correction tool served for drift correction; the E-z axis was stabilized via the “definite focus” system. PALM image calculation was performed applying the 2D x/z Gaussian model (Zeiss) using a peak mask size of 9 pixels, where one pixel corresponds to 100 nm and a peak intensity to noise ratio of 6. To exclude background and events resulting from the co-emission of co-localizing molecules, events were filtered for photon numbers between 70 and 350, and PSF (point spread function) width at 1/e maximum (70–170 nm) were employed as a last step, events were grouped according to the following parameters: three on-frames with 0 offset-frames allowed and a search radius of 30 nm.

When imaging strains containing ParB-PamCherry, four imaging series were taken for each field-of-view, where each subsequent series was characterized by a separate laser line (305 nm linear laser grid) and the other laser line (561 nm linear laser grid) and a laser line grid (0.01% to 1.0%, 0.1% to 1%, and 1% to 10%). Every other imaging parameter remained the same in between the time series. The frame count for each collection was 10,000 frames and converted molecules were imaged using the 561 nm laser at 15% (transfer mode) for 50 ns at a 200-fold EMCCD gain. For MksB-PamCherry, the 488 nm laser was turned off and the default laser line grid was applied. Therefore, the other imaging parameters were kept the same as in the case of ParB-PamCherry. DivIVA-mNeonGreen was imaged for 10,000 frames using the
488 nm laser at 30% (transfer mode) for 50 ms at 200 EMCCD (electron multiplying charge-coupled device) gain. In this case no 405 nm laser was used, as the fluorescence is not photoactivatable. At 488 nm laser excitation activation of PAmCherry, MkbB-PAmCherry was imaged prior to DivVA-mNeonGreen.

The workflow of protein cluster analysis is illustrated in Supplementary Fig. 7. The field-of-view in the bright-field channel was correct for illumination unevenness by dividing the field-of-view containing the cells of interest with an empty one (Process-Calculator Plus, Fiji) and enlarged ten times (bicubic interpolation). The resulting image was thresholded (Image-Adjust-Threshold) with default parameters and converted to a binary mask. A Fiji macro was then run on the binary mask to close the mask holes present within cells and to enlarge the cells mask themselves. Cells that were in contact with each other were separated via water shimming. The perimeter coordinates corresponding to masks representing cells lying within the focus were extracted and used to exclude events originating from cells lying outside the focal plane and the background. The clustering structures of events within a cell were identified via the OPTICS algorithm in R110. OPTICS is a clustering algorithm based on two parameters: minimum points (MinPts—in this case, a point is an event) and epsilon (ε—maximum search radius). As, in our case, only events within the same cell can belong to the same cluster, ε was chosen so that it would include all the events present within each cell (ε = 3000 nm). The effect of the MinPts value on the visualization of the cluster-ordering structure is shown in Fig. 8 through the ε=500 µm reaching such phenomenon. Specifically, 50 and 35 nm were chosen as thresholds, as, in the tested conditions, they were able to consistently identify and separate subclusters from the macroclusters they were lying into (Supplementary Fig. 7).

Documentation is available at Github (https://github.com/GiacomoGiacomelli/ParB-clustering-protein-profilng).

Chromosome conformation capture libraries. 3C/Hi-C libraries were generated as previously described by Val et al.111 with minor changes. Briefly, cells were grown in 200 ml of M9 minimal medium at 30 °C to an O.D. of 3 and rediluted to a final concentration of ~1 × 10^9 cells/ml. Cells were crosslinked using fresh formaldehyde for 30 min at RT (3% final concentration; Sigma Aldrich Formalin 37%) followed by 30 min at 4 °C. Formaldehyde was quenched using a final concentration of 0.25 M glycine for 20 min at RT. Cells were then collected by centrifugation, frozen in dry ice, and stored at −80 °C until use. Frozen pellets of ~10^9 cells were thawed on ice and suspended in a final volume of 1.1 ml 1× TE (pH 8) and transferred in a VK01 Preacell Tube (beads bearing). Fixed cells were disrupted using the following protocol on a precellys apparatus: 9 cycles × [20°→3500 rpm; 30°→pause] for each cycle. Lysate was transferred to a 1.5 ml tube, SDS 10% was added to the final concentration of 0.5% and the mix was incubated for 10 min at RT. 1 ml of lysate was then transferred in a 5 ml tube containing 4 ml of digestion mix (1× NEB 3 buffer, 1% Triton X-100, and 1,000 U MluCI enzyme). DNA was digested for 30 min at 37 °C under shaking. Insoluble DNA was removed by centrifugation, frozen in dry ice, and stored at −80 °C until use. DNA was precipitated using 1/10th volume of 3 M Na-Acetate (pH 5.2) and one volume of isopropanol. After 1 h at −80 °C, DNA was pelleted, resuspended in 900 µl 1× TE buffer, and extracted with 900 µl phenol–chloroform pH 8.0. DNA was again precipitated using 1/10th volume of 3 M Na-Acetate (pH 5.2) and 2.5 volume of cold Ethanol. Finally, DNA was resuspended in 100 µl 1× TE buffer supplemented with RNase and incubated 30 min at 37 °C. 3C libraries were then processed as described111 and paired-end sequenced on an Illumina NextSeq apparatus (2 × 35 bp). DNA content per cell was further determined by flow cytometry, yielding an average number of six chromosome equivalents per cell for all strains analyzed by Hi-C like approaches (Supplementary Fig. 2B, C and Supplementary Table 2).

Contact map generation. Contact maps were generated as previously described111. Read lengths were assigned independently (forward and reverse) using Bovine 2 in local and very sensitive mode and were assigned to a restriction fragment. Non-informative events (self-circularized DNA fragments, or unfragments) were discarded by taking into account the pair-reads relative directions and the distribution of the different configurations as described in Courmac et al.111. We then bin the sequences into 5 Kb macromaps to generate contact maps to visualize them using the sequential component normalization procedure111. Contact maps were then generated using Pyplot library and a saturation threshold at 99.5% of the maximum value.

Contact map comparison. Ratio between contact maps was computed for each point of the map by dividing the amount of normalized contacts in one condition by the amount of normalized contacts in the other condition and by plotting the log2 of the ratio. The color code reflects a decrease or increase of contacts in one condition compared to the other (blue or red signal, respectively). No change is represented by a white signal. To further compare the Hi-C data, we applied the HIReP software102 at a resolution of 5 Kb with a smoothing index of 3.

Identification of domains frontiers using directional index. To quantify the degree of directional preference, we applied on correlation matrices the same procedure as in Marbouty et al.111. For each 5 Kb bin, we extracted the vector of interactions from the correlation matrix between the studied bin and bins at regular 5 Kb intervals, up to 250 Kb in left and right directions. The two vectors were then compared with a paired t-test to assess their statistical significant difference (p = 0.05). The directional preferences for the bin along the chromosome are represented as a bar plot with positive and negative t-values shown as red and green bars.

Flow cytometry. DNA contents per cell were verified in C. glutamicum strains analyzed by 3C using flow cytometry as described before111. Flow cytometry analysis was performed as described before111. In short, exponentially growing cultures were treated with 25 µg/ml chloramphenicol for more than 4 h, to induce replication runouts. Cells were fixed in 70% ethanol (1:9 v/v) and washed once in PBS. Cell integrity was stained with SYBR® Green I (Invitrogen, 1:10,000 dilutions) for 15 min in the dark. Flow cytometry analysis was carried out subsequently using a BD Accuri C6 (BD Biosciences) equipped with a 488 nm laser. At least 200,000 events were collected per sample at a slow flow rate of 10 µl/min measuring ~5000 events per second, applying an acquisition threshold of 650 set on the green channel FL1-H. Data analysis was performed using the BD Accuri C6 Plus software (BD Biosciences). At first, events derived from cell aggregates were identified in plots of SSC vs. width and excluded from DNA content analysis. Remaining events were plotted as histograms vs. DNA amount (FL1-A, EM BP 533/30) at log scale, where chromosome numbers were assigned in accordance to calibration standards described before111. All experiments were performed in biological triplicates.

Comparison of contact signals with transcriptional data. RNA-sequencing data for C. glutamicum were recovered from ENA (Project PRJEB47788). Only reads with a mapping quality above 30 were conserved. Raw signal was then binned to match the binning of the corresponding contact maps and plotted along the genome. Both contact and transcription signals were smoothed with a Savitzky-Golay filter as previously described in Lioy et al.111.

Statistics and reproducibility. Correlation coefficients, linear regressions and analyses of nearest neighbor distance distributions were calculated using Excel 2019, Graph Pad Prism (GraphPad Software), and R (R-studio v1.4.3, R version v3.5.0). Micrographs contained in the main and supplementary figures are exemplary images from three biological replicates. Plasmid extractions in Fig. 5f were repeated three times; control western blotings in Supplementary Fig. 8A were performed once, and gels in Supplementary Fig. 12B are exemplary data from two replicates.

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

Data availability C. glutamicum ATCC 13032 genome sequence was obtained from GenBank (GenBank:DSX92741.7). RNA-seq data for C. glutamicum were recovered from ENA (Project PRJEB47788). Protemic data are available via ProteomeXchange with the project identifier PXD008896 (ref. 111). Genome-wide sequencing reads of CHIP-seq and chromosome conformation capture assays generated in this study are available in the Sequence Read Archive (SRA) under accession numbers PRJNA529385 and PRJNA552583. Flow cytometry raw data results were deposited in the FlowRepository database (accession number FR:FCM-Z2DJ112). The source data underlying Figs. 2c, h, 3b, d, e, and 5b, e, f and Supplementary Figs. 2B, C, 3B–D, 5A, 7C, 7E, 8A–C, 9B–D, 10A, B, 12A, B, D, and 13A, C are provided as a Source Data file.

Code availability The custom computer algorithm used in the analysis of PALM data has been deposited in Github (https://github.com/GiacomoGiacomelli/ParB-clustering-protein-profilng).

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**Author contributions**

Conceptualization: K.B., M.M., R.K., and M.B. Methodology—Strain constructions and analyses, epifluorescence microscopy, ChIP, bacterial two-hybrid screening, in-vitro protein assays: K.B.; Chromosome conformation capture: M.M.; PALM: G.G.; Mass spectrometry: A.S. and A.I. Writing manuscript: K.B., M.M., A.S. (Methods), G.G. (Methods), R.K., and M.B.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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