Supplemental material

Novel chromosome organization pattern in actinomycetales–overlapping replication cycles combined with diploidy

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Table S1. Oligonucleotides used in this study.

Oligonucleotides Strain constructions	Sequence 5'–3'	Restriction sites [a]
ParB-Hind-up-F	CATAAGCTTAGCTGAATCCTTTGGAAG	HindIII
ParB-Sal-up-R	CATGTCGACTTGGCCCTGGATCAAGGA	SalI
ParB-XbaI-D-F	CATTCTAGATAATTTTAAGTTTGGCGC	XbaI
ParB-Bam-D-R	CATGGATCCCCTCCACATCAATCAGGC	BamHI
eYFP-SalI-F	CATGTCGACATGGTGAGCAAGGGCGAG	SalI
eYFP-XbaI-R	CATTCTAGACTTGTACAGCTCGTCCAT	XbaI
mCherry2-SalI-F	ATAGTCGACATGGTCAGCAAGG	SalI
mCherry2-XbaI-R	ATATCTAGAGGATCCTGAGCCG	XbaI
ParB-N-ter-SalI-F	CAGGTCGACATGGCTCAGAACAAGGGTTCC	SalI
ParB-PstI-800D-R	CAGCTGCAGCCAACCCCGATGACCTGG	PstI

DnaN-Hind-up-F	CATAAGCTTGGTTGGCCGCGAAGGACT	HindIII
DnaN-SphI-up-R	CATGCATGCGCCTGGCAGGCGCACTGG	SphI
DnaN-XbaI-D-F	CATTCTAGATAAACACAAAAGTTTCAC	XbaI
DnaN-BamHI-D-R	CATGGATCCTCTGCTGGCTCGCCTTTG	BamHI
mCherry-SalI-F	CATGTCGACATGGTGAGCAAGGGCGAG	SalI
mCherry-XbaI-R	GCGTCTAGATTACTTGTACAGCTCGTC	XbaI
DnaN-N-ter-F	ATGGAGTCACAAAACGTGTCCTTC	
DnaN-Bam-700D-R	CATGGATCCGGCGTGCCAACTGG	BamHI

Oligonucleotides Marker frequency analysis	**Sequence 5'–3'**	**Organism and chromosomal binding region**
Cg0002-165-F	TTTTGGGGAGTTGTGCACAG	*C. glutamicum*, cg0002
Cg0002-165-R	GGGTTGTGCAGGGATTTTGT	*C. glutamicum*, cg0002
Cg1702-159-F	TGAAGCTATCCTCAACGGCA	*C. glutamicum*, cg1702
Cg1702-159-R	TAACCAATCGCGATGCCTTG	*C. glutamicum*, cg1702
DivIVA-153-F	CTACAACGAAGACGAGGT	*C. glutamicum*, cg1702
DivIVA-153-R	GCAGTTGAGGAACTAGCA	*C. glutamicum*, cg0018
Cg0018-262-F	CCTGCTCAGAATGAAACC	c *C. glutamicum*, g0018

Cg0018-262-R	AGCTGCTACTACTTGGGC	*C. glutamicum*, cg0018
Bsu-oriC-F	GATCAATCGGGGAAAGTGTG	*B. subtilis*, 5’ site: BSU00010, 3’ site: BSU00020
Bsu-oriC-R	GTAGGGCCTGTGGATTTGTG	*B. subtilis*, 5’ site: BSU00010, 3’ site: BSU00020
Bsu-terC-F	TCCATATCCTCGCTCCTACG	*B. subtilis*, 5’ site: BSU19200, 3’ site: BSU19210
Bsu-terC-R	ATTCTGCTGATGTGCAATGG	*B. subtilis*, 5’ site: BSU19200 3’ site: BSU19210

11 [a] Restriction sites are underlined.

12

Table S2. Bacterial strains and plasmids used in this study.

Strain	Characteristics	References/ Source
E. coli DH5α	F^- ϕ80*lacZ*ΔM15 (*lacZYA-argF*)*U169 recA1 endA1 hsdR17*(r_K^- m_K^+) *supE44 phoA thi-1 gyrA96 relA1* λ^-	Invitrogen
B. subtilis 168	*trpC2*	Laboratory collection
***C. glutamicum* RES 167**	Restriction-deficient mutant, otherwise considered wild type	(1)
C. glutamicum parB::parB-eYFP	RES167 derivative, *parB-eYFP*	This study
C. glutamicum parB::parB-mCherry2	RES167 derivative, *parB-mCherry2*	This study
C. glutamicum ΔparA	RES167 derivative, *ΔparA*	(2)
C. glutamicum ΔparA parB::parB-eYFP	RES167 derivative, *ΔparA, parB-eYFP*	This study
C. glutamicum divIVA::divIVA-mCherry	RES167 derivative, *divIVA-mCherry*	(3)
C. glutamicum divIVA::divIVA-mCherry parB::parB-eYFP	RES167 derivative, *divIVA-mCherry*, *parB*-eYFP	This study
C. glutamicum dnaN::dnaN-mCherry	RES167 derivative, *dnaN-mCherry*	This study
C. glutamicum parB::parB-	RES167 derivative, *dnaN-*	This study

eYFP dnaN::dnaN-mCherry	*mCherry*, *parB-eYFP*	
Plasmids	**Characteristics**	**Reference/ Source**
pK19mobsacB	Integration vector, *ori* pUC, Km^r, *mob sac*	(4)
pK19mobsacB-parB-eYFP	Integration vector, *ori* pUC, Km^r, *mob sac, parB-eYFP*	This study
pK19mobsacB-parB-mCherry2	Integration vector, *ori* pUC, Km^r, *mob sac, parB-mCherry2*	This study
pK19mobsacB-DnaN-mCherry	Integration vector, *ori* pUC, Km^r, *mob sac, dnaN-mCherry*	This study

14

15

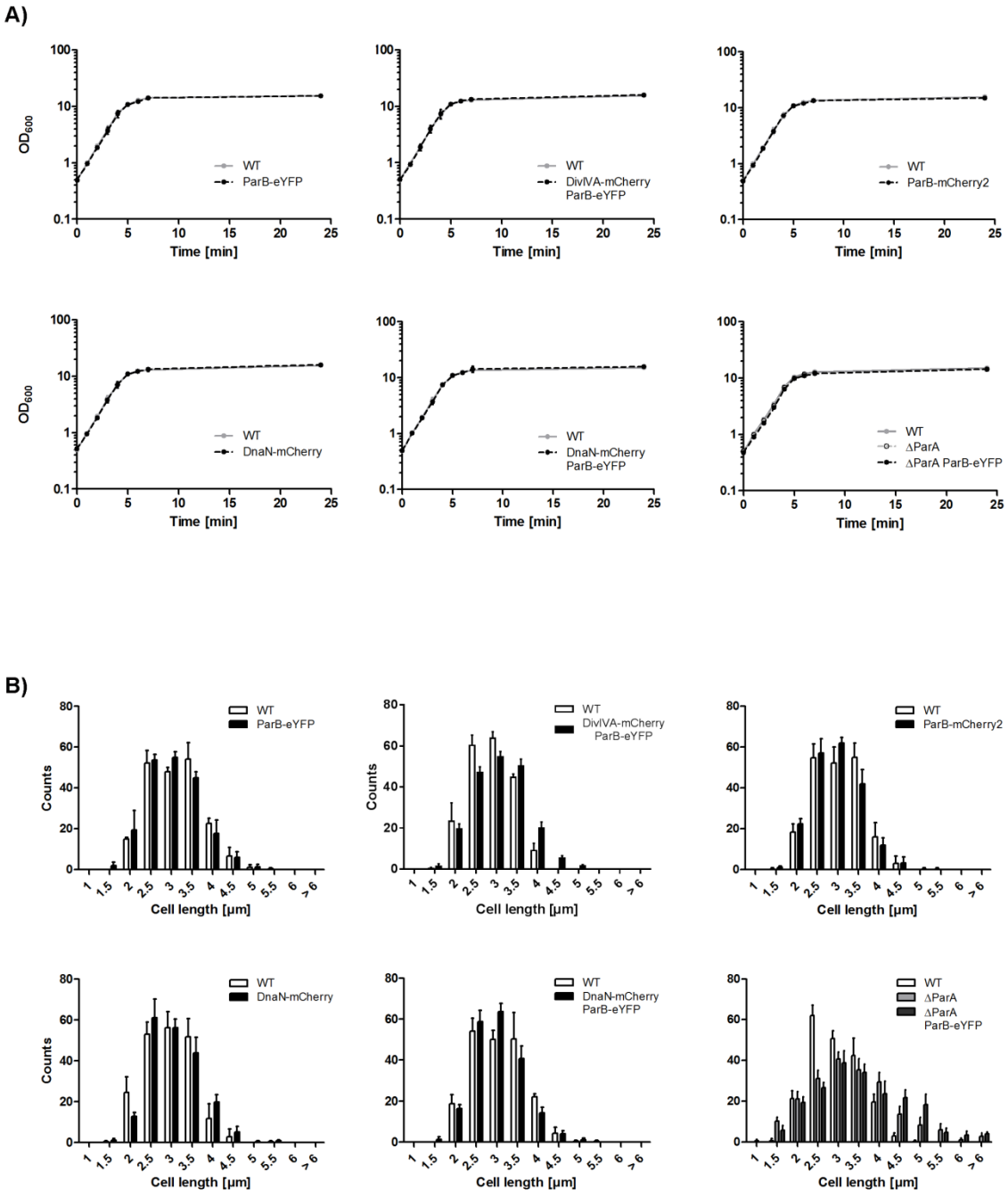

Figure S1. Growth and cell length distributions of strains used in this work.

Comparison of **A)** growth curves and **B)** cell length distributions (n=200) of *C. glutamicum* mutant strains with wild type. All growth experiments were performed in

BHI medium. Values derive from triplicates; standard deviations are indicated in all graphs. Mutant strains analyzed are: *C. glutamicum parB::parB-eYFP*, *parB::parB-eYFP divIVA::divIVA-mCherry*, *dnaN::dnaN-mCherry*, *parB::parB-mCh2*, *parB::parB-eYFP dnaN::dnaN-mCherry*, *ΔparA* and *ΔparA parB::parB-eYFP*.

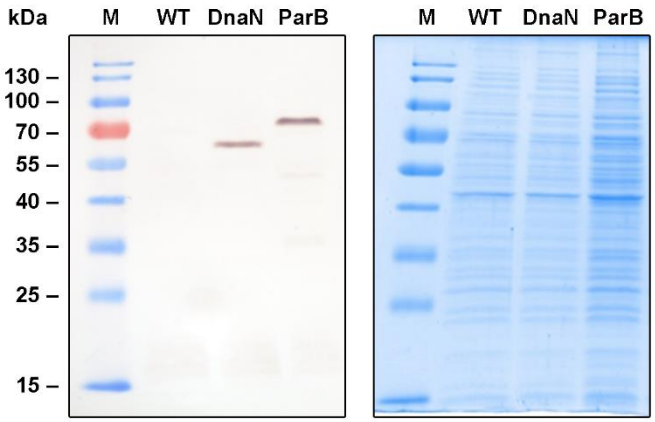

Figure S2. Validation of full-length fusion proteins ParB-mCherry2 and DnaN-mCherry.

Full length fluorescent fusion proteins were validated via western blotting. Whole cell lysates of *C. glutamicum* wild type, *dnaN::dnaN-mCherry* and *parB::parB-mCh2* strains were probed and DnaN-mCherry and ParB-mCherry2 were detected via polyclonal α-mCherry antibody. Shown are western blot (left) and corresponding coomassie stained gel (right).

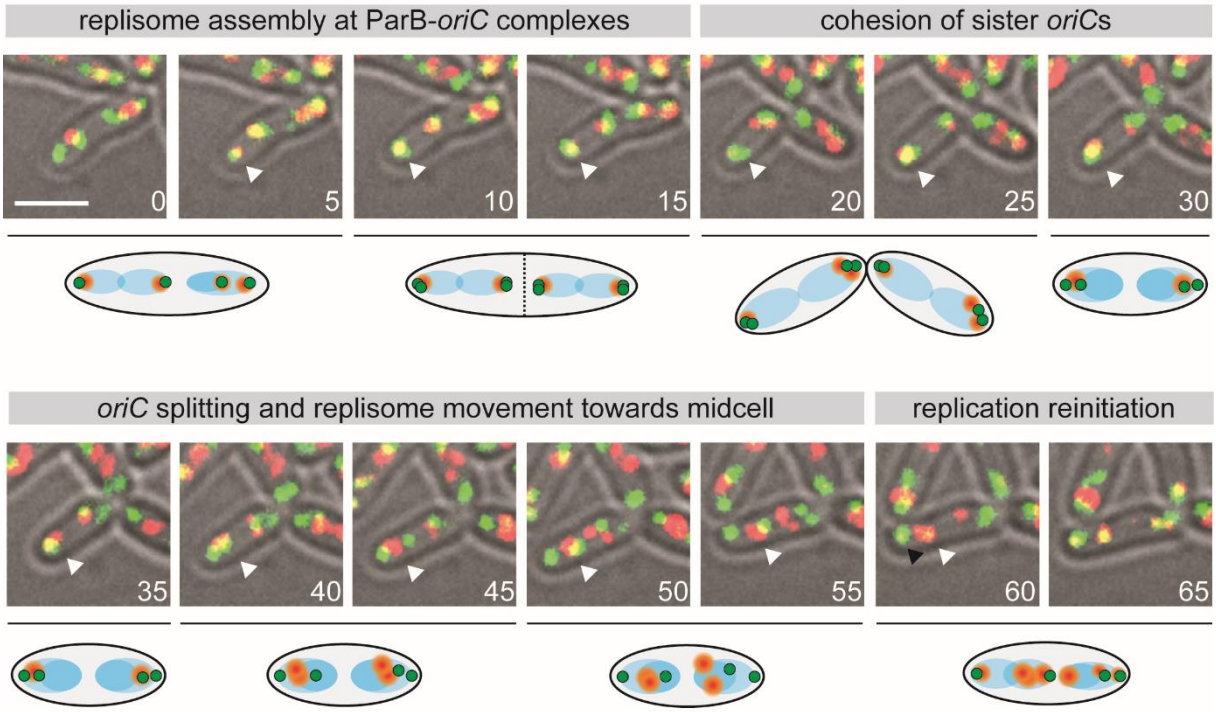

Figure S3. Single cell analysis of replisome dynamics.

Replication cycle accounting for gradual replisome movement towards midcell. Time series exemplifiying movement of replisomes relative to *oriC*-ParB complexes (green and red, overlay in yellow) in a strain encoding ParB-eYFP and DnaN-mCherry as allelic replacements of the native genes. Images were taken in 5 min intervals (bottom right corner). At time point 5 polar replisomes assemble in a pre-divisional cell (white arrowheads), where newly replicated sister *oriC*s colocalize to time point 40; replisomes gradually move away from polar *oriC*. Prior to replication termination a new round of replication is initiated at time point 60 (black arrowhead). Sister replisomes merge in one fluorescent spot, but frequently split (time point 50 and 55, see cartoon below images). Scale bar, 2 µm.

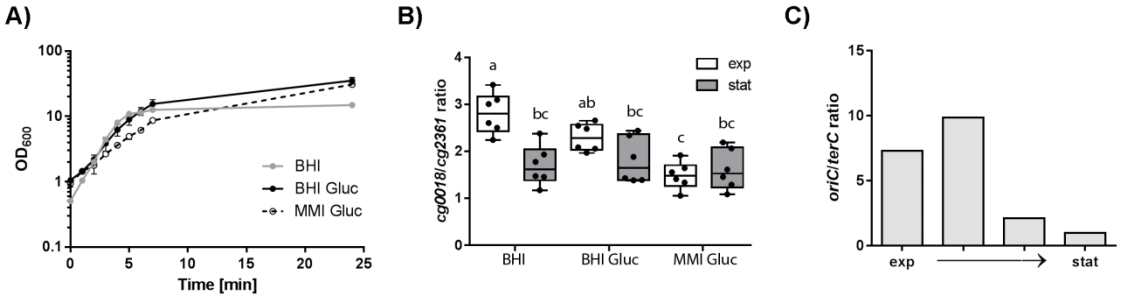

Figure S4. Examination of replication initiation.

A) Growth curves of *C. glutamicum* wild type strain grown in BHI, BHI supplemented with glucose or MMI supplemented with glucose. Genomic DNA was isolated in exponential and stationary growth phases and utilized in marker frequency experiments and for whole genome sequencing. Values derive from triplicates; standard deviations are indicated in all graphs. For growth rates see Table 1. **B)** *OriC* to terminus ratios via marker frequency analysis of chromosomal loci *cg0018* and *cg2361*; Fig. 5A). An ANOVA revealed an effect of growth phase ($F(1,30)=13.41$, $p<.001$), of medium used ($F(2,30)=9.77$, $p<.001$) and significant interaction of growth phase and medium ($F(2,30)=7.17$, $p<.01$). **C)** Marker frequency analysis of *Bacillus subtilis* 168 genomic DNA (marker in intergenic *oriC* and *terC* regions) isolated from cells grown in LB medium at time points of exponential (*oriC*/*terC* >10) to stationary growth (*oriC*/*terC* <2).

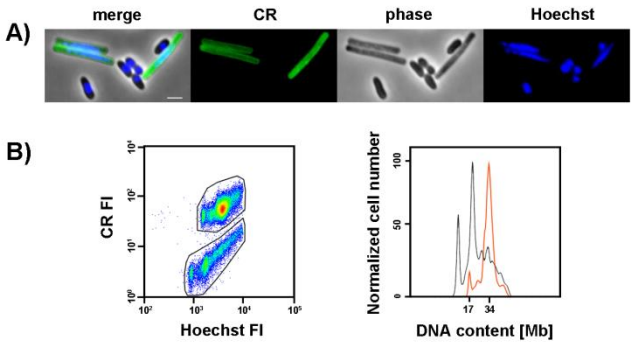

Figure S5. Calibration of DNA measurements.

B. subtilis cells of known DNA content served as internal standard in flow cytometry measurements after applying SPAAC cell wall staining with Carboxyrhodamine. The standard was included in samples of *C. glutamicum* cells grown at different rates; DNA was stained with Hoechst. **A)** Flow cytometry sample of *B. subtilis* and *C. glutamicum* cells. Shown is the overlay (merge) of Carboxyrhodamine (green) and Hoechst fluorescence (blue) with the phase contrast image and individual channels (CR, phase, Hoechst). Scale bar, 2 µm. **B)** Gating strategy exemplified on sample containing BHI-grown *C. glutamicum* cells. Left: *C. glutamicum* and *B. subtilis* subpopulations were identified in density plots of the Carboxyrhodamine (CR FI, a.u.) vs. the Hoechst channel (Hoechst FI, a.u.) according to their green fluorescence intensity. Right: Determination of *C. glutamicum* chromosome numbers (black) according to the *B. subtilis* standard (red) in histograms vs. DNA content.

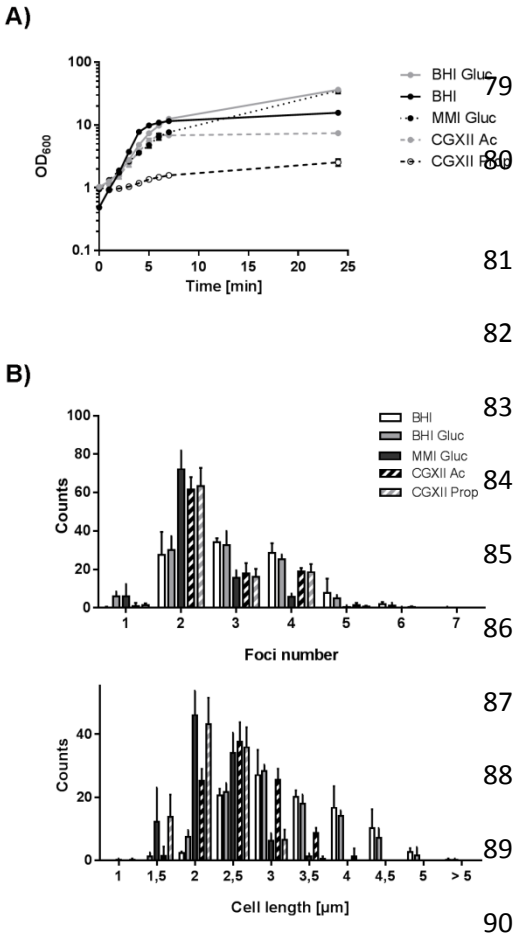

Figure S6. Medium dependency of *oriC*-ParB cluster numbers per cell.

Comparison of **A)** growth and **B)** distribution of cell lengths and ParB-eYFP cluster numbers (n=200) of strain *parB::parBeYFP* at use of different carbon sources. Cells were grown in BHI (µ=0.62), BHI (µ=0.49) or MMI supplemented with glucose (µ=0.32), and CGXII supplemented with Acetate (µ=0.41) or Proprionate (µ=0.13). Experiments were performed in triplicates; error bars display standard deviations.

Movie S1: Growth of *C. glutamicum parB::parB-eYFP* cells in a microfluidic chamber with image acquirements every 5 min for a total of 225min (AVI format).

Movie S2: Growth of *C. glutamicum dnaN::dnaN-mCherry* cells in a microfluidic chamber with image acquirements every 5 min for a total of 230min (AVI format).

References

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2. Donovan, C., Schwaiger, A., Krämer, R. and Bramkamp, M. (2010) Subcellular localization and characterization of the ParAB system from *Corynebacterium glutamicum*. *Journal of bacteriology*, **192**, 3441-3451.
3. Donovan, C., Sieger, B., Krämer, R. and Bramkamp, M. (2012) A synthetic *Escherichia coli* system identifies a conserved origin tethering factor in Actinobacteria. *Molecular microbiology*, **84**, 105-116.
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