Expanded View Figures

A

B

Figure EV1. GapR homologs.

A Shown is a protein phylogenetic tree composed of representative members of DUF2312 (pfam10073) found in archaea, eukarya, bacteria, and phages. See Appendix Supplementary Methods for tree construction.

B Examples of *gapR* homologs in the genome of phages CR30ϕ and RDJLϕ1 in which *gapR* homologs are located close to genes encoding the sliding clamp and the DNA polymerase, respectively.

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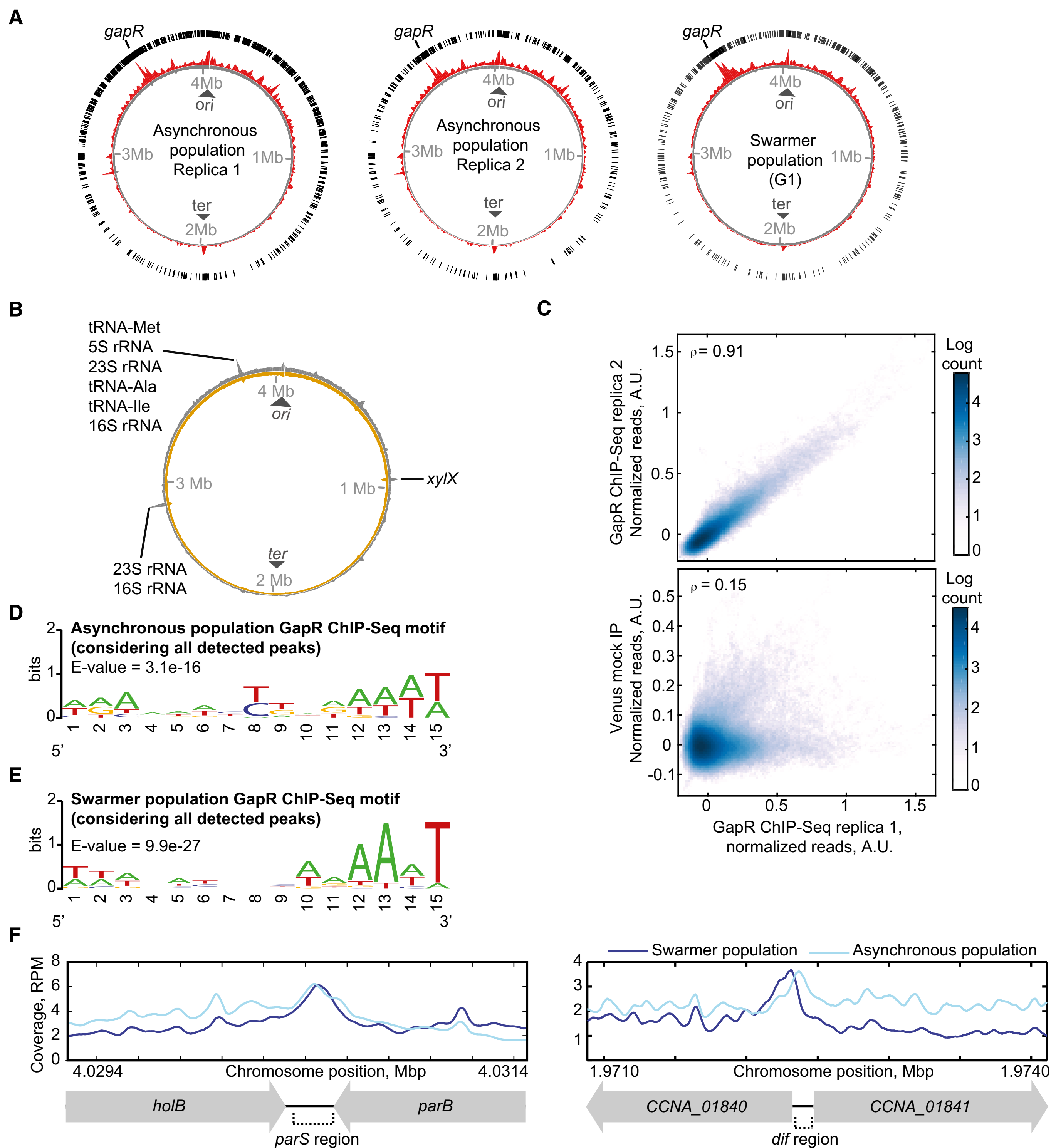

Figure EV2.

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◀ **Figure EV2. ChIP-seq data analysis for GapR-Venus and controls.**

A Whole-genome profile of GapR-Venus normalized ChIP-seq read counts for two replicates of asynchronous populations (left and middle panels) and for a swarmer population (right panel) of strain CJW5534. The circle with exterior black bars shows the distribution of ChIP-seq peaks detected with MACS2 software. The inner red circle shows the normalized ChIP-seq read counts. The gray inner circle shows the level of total DNA before immunoprecipitation by GapR-Venus. The location of the *gapR* locus in the *Caulobacter crescentus* chromosome is indicated.

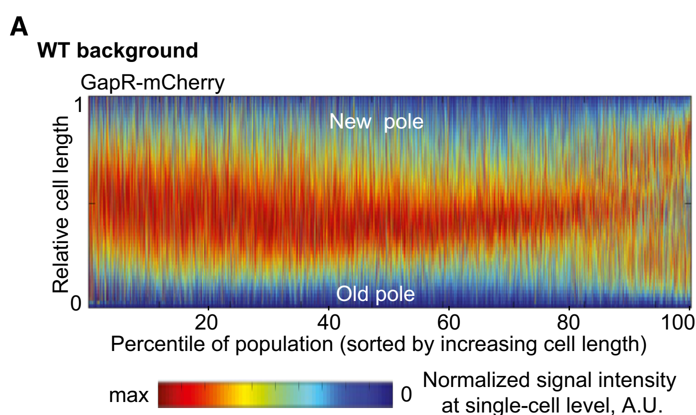
B Whole-genome profile of normalized ChIP-seq read counts for mock immunoprecipitation performed with the freely diffusing Venus protein expressed at the *xylX* locus (strain CJW5796). Exterior gray circle shows normalized ChIP-seq read counts following immunoprecipitation with Venus. Light brown inner circle shows the level of total DNA before immunoprecipitation by Venus. The location of ribosome- and tRNA-encoding loci and of the *xylX* locus is indicated.

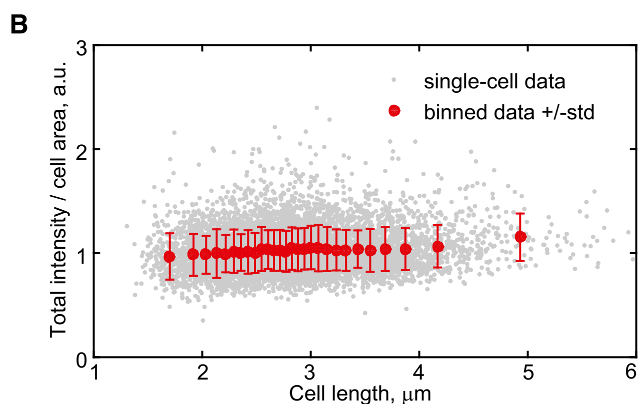
C Pearson correlation at the single-nucleotide level in normalized read counts between two independent replicates of the GapR-Venus ChIP-seq experiment and between GapR-Venus ChIP-seq and the mock immunoprecipitation with freely diffusing Venus.

D Best MEME consensus-sequence motif for GapR binding using ChIP-seq data from an asynchronous cell population. For this analysis, all peaks were considered, including peaks detected at ribosomal, tRNA, and *gapR* loci.

E Same as (D) but from a swarmer cell population.

F GapR-Venus ChIP-seq coverage at the *parS* and *dif* regions.





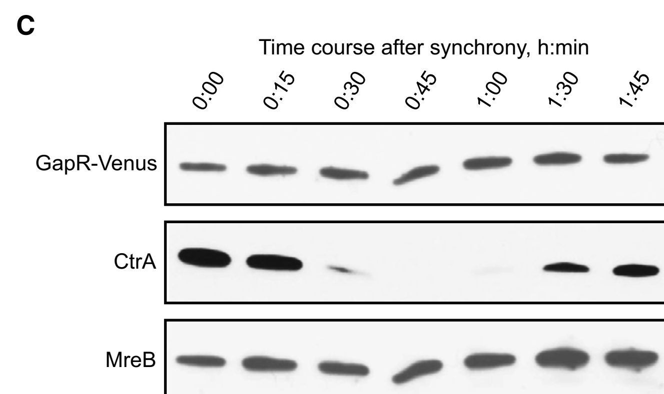


Figure EV3. Cell cycle dynamics of GapR localization are not caused by changes in GapR protein levels.

A Demograph of an asynchronous population (n = 4,266 cells) showing the cell cycle localization of GapR-mCherry (strain CJW5810), shown in absolute cell-length scale. Cells were sorted by increasing cell length, and cell coordinates were oriented using new-pole marker TipN-GFP.

B Fluorescence intensity of GapR-Venus (total intensity divided by cell area) for cells represented in the demograph shown in (A; strain CJW5810, top panel). Cells were sorted by length as in (A). Gray dot = single-cell value. Red dot and bar = mean ± standard deviation value for 300 cells.

C Western blot of cell lysates collected every 15 min from a synchronized cell culture of CJW5534, in which GapR-Venus is expressed from its native chromosomal locus and promoter. CtrA and MreB were used as cell-cycle-synchronization and loading controls, respectively. See Appendix Supplementary Methods for Western blot experimental conditions.

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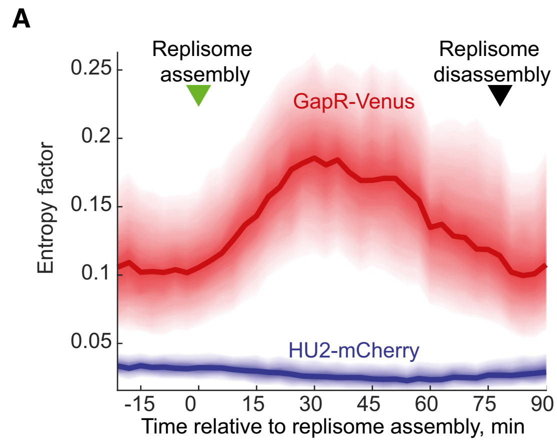

Figure EV4. Population analysis of GapR and HU2 spatial organization during the cell cycle.

Plot showing how the spatial distribution of GapR-Venus and HU2-mCherry deviates from their theoretical maximum entropy values, with the greater the values are, the more organized (the least dispersed) the signal is. In this plot, maximum entropy (uniform distribution) corresponds to an organization factor value of zero. The data were obtained from time-lapse experiments of CJW5932 (n = 752) or CJW5969 (n = 579) cells and were computationally synchronized by maximizing the correlation in DnaN-CFP signal dynamics. See Code EV3 information for details about the calculation of the organization factor.

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