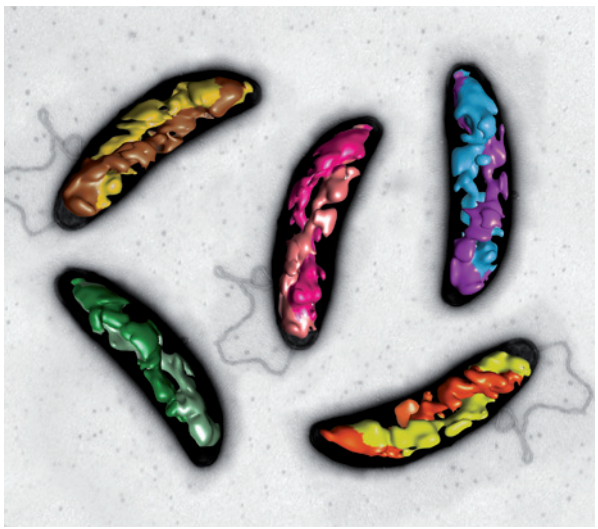
BACTERIAL PHYSIOLOGY

Seeing *Caulobacter* in 3D

What are the factors that determine the global conformation and architecture of a bacterial genome? In a recent *Molecular Cell* paper, a comprehensive three-dimensional analysis of the *Caulobacter crescentus* genome provides some answers to this fundamental question.

The origin and terminus of replication in *C. crescentus* swarmer cells are located at opposite cell poles, with the two arms of the chromosome running parallel to the long axis of the cell. Mark Umbarger, Esteban Toro and colleagues used a modified chromosome conformation capture (3C) method to describe the 3D architecture of the *C. crescentus* swarmer cell genome. 3C is a method for mapping local and long-range chromosomal interactions. First, the genome is treated with formaldehyde, which crosslinks genomic regions that are in close physical proximity to each other. Restriction enzyme digestion of the genome creates crosslinked fragments that can be ligated, and the ligation frequency of two loci then provides a measure of the distance between them. 3C carbon copy, or 5C, allows analysis of interactions on a larger scale and involves highly multiplexed ligation-mediated amplification followed by high-throughput sequencing.



Three-dimensional representations of the *Caulobacter crescentus* genome, with the two chromosome arms in contrasting colours. Image courtesy of M. A. Umbarger, Harvard Medical School, Boston, USA, and E. Toro, Stanford University, Palo Alto, USA.

Umbarger *et al.* applied the 5C technique to a population of synchronized *C. crescentus* swarmer cells and used the resulting distances to computationally derive high-resolution (~13 kb) 3D genome models. These models indicated that the *C. crescentus* chromosome is ellipsoidal in shape, with the origin and terminus located at opposite cell poles and the two chromosome arms probably intertwined. The model also correctly predicts that the two *parS* sites, which are involved in *C. crescentus* chromosome segregation, are located near the origin-proximal pole. To probe the importance of *parS* positioning in defining the organization of the genome, the authors performed 5C analysis on a *C. crescentus* inversion strain (ET166) in which a 10 kb region containing the *parS* sites was shifted ~400 kb from the origin. The results showed a massive reorientation of the entire genome that retained the polar location of the *parS*-containing region. No reorganization was observed in a control strain which contained a similar sized inversion that did not move the *parS* sites. The clockwise rotation of the entire genome in *C. crescentus* ET166 was confirmed *in vivo* using live-cell imaging, which also revealed that the terminus is free to rotate, indicating that the only fixed point is the origin. Perhaps surprisingly, this large-scale rearrangement had little overall effect on gene expression. Finally, analysis revealed that the ~100 kb centromeric region flanking the *parS* sites displays a compact chromatin conformation, which the authors speculate could reflect the actions of the chromosome partitioning protein ParB and structural maintenance of chromosomes (SMC) proteins.

This approach provides direct structural evidence of the importance of the *parS* sites for global positioning of the *C. crescentus* genome. Although such analysis is technically challenging, the authors suggest that this approach could be used to study the role of specific chromosomal elements in dynamic genome organization in other bacterial and eukaryotic systems.

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ORIGINAL RESEARCH PAPER Umbarger, M. A. *et al.* The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. *Mol. Cell* **44**, 252–264 (2011)