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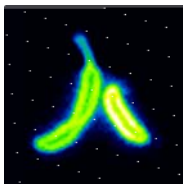
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Coordination of chromosome replication, segregation and cell division in *Caulobacter crescentus*

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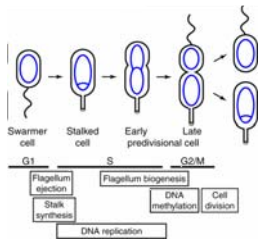


Abstract

In *Caulobacter crescentus*, progression through the cell cycle is coupled to a cellular differentiation program. Here, the terminus region of the *Caulobacter* chromosome were identified and the temporal coordination between multiple different cell cycle events was characterized. A very short delay between initiation of DNA replication and origin movement is observed, indicating absence of cohesion between newly replicated chromosomes. However, completely replicated terminus regions stay associated until shortly before cell division. Cell constriction take place over a non-separated nucleoid, indicating absence of nucleoid occlusion of cell division in *Caulobacter*.

Introduction

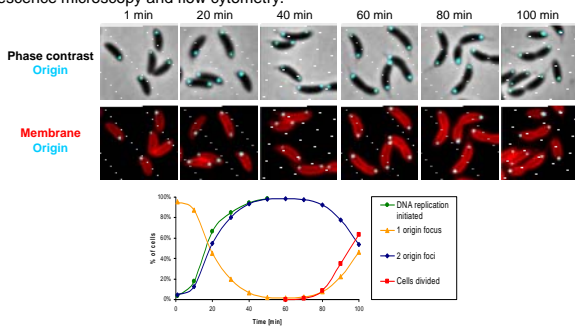
Caulobacter crescentus exists as morphologically and functionally distinct cell types and cellular differentiation is an integral part of its cell cycle (1). Asymmetric cell division gives two different progeny cells: A non-motile stalked cell with a cylindrical extension (a stalk) at one pole and a motile swarmer cell that possesses a single polar flagellum. The stalked cell initiates chromosome replication and cell elongation immediately after cell division, whereas the swarmer cell is unable to do so. After a defined period, the swarmer cell differentiates into a stalked cell by shedding the flagellum and synthesizing a stalk at the same pole. Chromosome replication is initiated during the swarmer-to-stalked cell transition. In the middle of the cell cycle, a new flagellum is synthesized at the pole opposite the stalk and cell division is initiated.



Origin movement

The swarmer cell possesses a single copy of the chromosome, which is oriented with the origin-proximal region located close to the flagellated pole of the cell. After initiation of DNA replication, one of the newly replicated origins moves to the opposite pole of the cell.

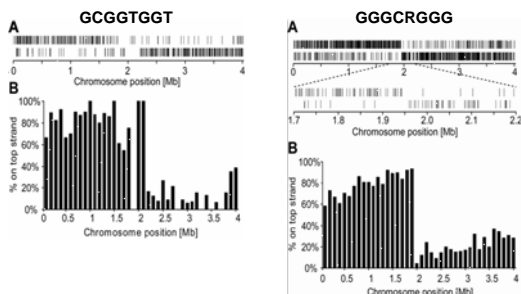
To characterize the coordination between DNA replication and chromosome movement, a strain where the origin-proximal region of the chromosome is tagged with CFP-LacI (2) was synchronized and the cells at the different stages of the cell cycle were characterized by fluorescence microscopy and flow cytometry.



A very short delay between initiation of DNA replication and origin movement is observed, indicating either absence of or only a very brief period of cohesion between the newly replicated origin-proximal parts of the *Caulobacter* chromosome.

Identification of the terminus region

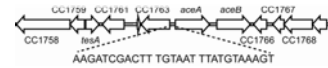
To identify the *Caulobacter* terminus region, a combined bioinformatics and experimental approach was used. Most bacterial genomes contains skewed sequences that abruptly switch strands near the origin and terminus. Two highly significantly skewed sequences were identified, that abruptly switch strand near position 1.95 Mb opposite the origin on the genetic map.



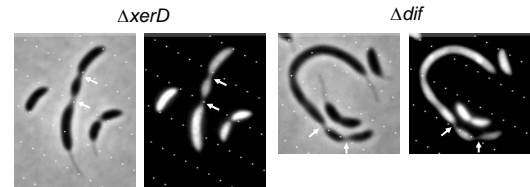
The GGGCRGGG motif is very similar to the DNA motifs from *E. coli* that control FtsK-mediated movement of the terminus-proximal part of the chromosome.

Identification of the dif site

A putative *dif* recombination site near the position where the GGGCRGGG motifs switch strands was identified.



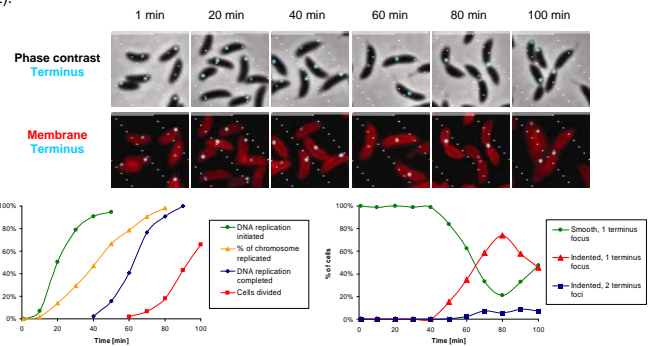
To verify that this site is the *Caulobacter dif* site, strains with deletions of the putative *dif* site, *xerC* and *xerD* were constructed.



Since the same chromosome segregation and cell division defect are observed in 2 - 4 % of the cells for all three strains, the site is the *Caulobacter dif* site. The site is unusual since it is located close to genes with functions in central metabolism. The XerC binding site in *Caulobacter dif* diverges very significantly from other known sites.

Terminus movement and separation

Coordination between cell cycle progression, terminus movement and separation was examined using a strain with the terminus region of the chromosome tagged with CFP-LacI (2).

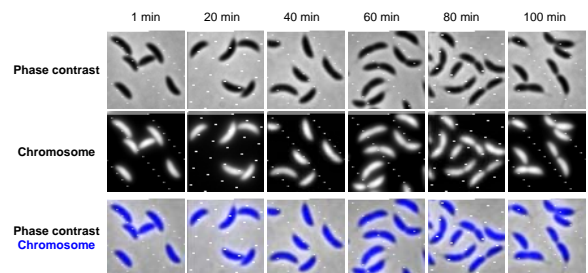


The terminus-proximal part of the chromosome gradually moves from the pole opposite the stalk to mid-cell during the DNA replication process. The completely replicated terminus regions stay associated with each other for an extended period of time after chromosome replication is completed. The terminus regions disassociate shortly before the dividing cells separate.

Chromosome trapping by the septum

Membrane staining show cytoplasmic membrane invagination before separation of the replicated terminus foci (see above).

To examine coordination between cell division and nucleoid separation, the chromosomal DNA in synchronized cells were stained using DAPI. Chromosomal DNA is present in the entire cell at all stages during the cell cycle, no DNA-free regions are observed.



Invagination of the cytoplasmic membrane is observed before the terminus regions separate and two nucleoids are formed, resulting in trapping of a chromosome on either side of the cell division septum. Thus, no nucleoid occlusion of cell division is observed in *Caulobacter*. Cytoplasmic membrane invagination takes place approximately 20 minutes before separation of the progeny cells, during the final step of the cell division process. The uncoupling between invagination of the cytoplasmic membrane and the outer part of the cell wall could be responsible for the previously observed compartmentalization of the predivisional cell.

References

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- 2 Viollier, P. H., Thanbichler, M., McGrath, P. T., West, L., Meevan, M., et al. (2004) *Proc Natl Acad Sci U S A* 101, 9257-9262.

Acknowledgements

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