Quantitative Analysis of DNA transport and storage during replication in *Caulobacter crescentus*

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Short Abstract — Caulobacter crescentus **has one circular chromosome that is approximately linearly stored in the cell at the time of cell division. This structure is established as newly-replicated DNA is segregated and stored during replication [1]. We simultaneously visualized multiple chromosomal fluorescent-tagged loci at various locations on the chromosome during the dynamic process of chromosome transport and storage. We found the DNA is initially compacted immediately after it emerges from the replisomes. This compacted DNA of the two new chromosomes moves rapidly toward opposite cell poles where it is compacted further. This suggests multiple compacting mechanisms are used in this dynamic process.**

I. Background and Approach

Caulobacter has a 1.2mm long circular chromosome that is compacted and stored in each of the ~1.5 μm long daughter cells when the cell divides. Each chromosome is organized with the origin of replication tethered at the old cell pole and the terminus near the new pole [2, 3, 4]. The DNA is replicated bi-directionally from the origin to the terminus. The two new chromosomes are moved rapidly towards the two cell poles on emergence from the replisomes. The chromosomes are positioned as they arrive at the poles and then further compacted. This transport process leads to corresponding loci in different cells being located along the cell approximately in proportion to their chromosomal distance from the origin of replication [1].

We used time lapse microscopy to observe positions of multiple fluorescent-tagged chromosomal loci as they moved from the replisome to their final position in wild type cells and in cells with deletions of proteins active in DNA compaction. By analyzing the resulting images we measured the speed of transport of the loci and the distance between two loci during transport.

II. Results

The DNA is compacted before it begins movement toward its destination pole. In different cells, the distance between two tagged loci during the movement phase was observed to vary between cells and to change with time during movement. The average physical distance between tagged loci was not proportional to the genomic distance between the loci. The distribution of these distances suggests that the compaction process is stochastic. Further, the inter-locus distance was positively correlated with the speed of segregation, suggesting the compaction is not rigid. In strains with deletion of one or both HU chromosomal compaction proteins, we see decrease in the compaction during movement compared to wild type strains. However, neither the speed of DNA movement nor the final compacted structure of the DNA was changed in HU deletion strains. This suggests that *Caulobacter* has other DNA compacting schemes active during both compaction for movement and final compaction of the DNA.

III. Conclusion

The DNA is compacted initially by multiple stochastic mechanisms starting as soon as the new DNA emerges from the replisomes. This initial compaction of the DNA is stochastic, non-rigid and HU dependent. The resulting degree of compaction of DNA segments is random, rather than uniform. DNA transport is also stochastic with instantaneous random variation in the strain on the DNA and the resulting instantaneous rate of movement.

References

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