Geometrical ordering of DNA in bacteria

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The bacterium Caulobacter crescentus shows a remarkable spatial ordering of its chromosome that leads to a strong linear correlation between the position of genes on the chromosomal map and their spatial position in the cellular volume. In a recent study we have shown that a robust and universal geometrical ordering mechanism can explain this correlation. We demonstrated that self-avoidance of DNA, specific positioning of one or few DNA loci (such as origin or terminus) together with the action of DNA compaction proteins (that organize the chromosome into topological domains) are sufficient to get a linear arrangement of the chromosome along the cell axis. This configuration, however, only represents the population average. Individual cells can have DNA arrangements that deviate significantly from the mean configuration and that break left-right symmetry. Symmetry breaking is stronger for longer chromosomes.

Recently it has been experimentally demonstrated that the genome of the bacterium C. crescentus has a highly regular spatial structure.1 In swarmer cells (that are in the non-replicating G1 state) origin (ori) and terminus (ter) are positioned at opposite cell poles. The intervening chromosomal loci show a strong linear correlation between their position on the chromosomal map and their position in the subcellular volume. Similar arrangements have been found in E. coli cells.2 However, here dynamics and organization of the chromosome are more complex and growth-phase dependent.3-9 Typically, localization patterns with ori and ter at opposite poles are only found in newborn cells.10,11

In a recent study,12 we have theoretically analyzed the basis of chromosomal organization in bacteria. We demonstrated that confinement of chromosomal domains to specific cellular positions has a strong influence on the spatial arrangement of the chromosome in the cell. In particular, we found that positioning of ori and ter to opposite cell poles in C. crescentus gives rise to the striking linear correlation found in reference 1. For E. coli we made predictions about the growth-stage dependence of the spatial arrangement of the chromosome. The conclusion were drawn from a theoretical model with the following main ingredients:

(1) All cells have a single chromosome of fixed length that lies inside the prescribed cellular volume. The origin and terminus have fixed spatial positions.

(2) The cellular volume is represented by a three-dimensional cubic lattice. The chromosome is represented by a self-avoiding random walk on this lattice.

(3) Each step of the random walk represents a compacted unit of the chromosome.

Compaction is the key ingredient of our model that is required to obtain the experimentally observed linear correlation. The specific scenario that we have in mind is that compaction proteins (such as H-NS, HU, FIS and TktA13) locally compact the chromosome giving it the shape of a chain of spheres (i.e., “blobs”) with a typical diameter d_b = 30 nm. Each step of the random walk represents such a blob. From the measurements of reference 14 we concluded that there are ~2,000 of these compacted units. A similar description of the chromosome was recently developed by Jun and coworkers in the context of...
The big advantage of our model is that it does not depend on the details on how these blobs are formed or even what they correspond to; the only requirement is that the blobs effectively reduce the length of the random walk. In particular, our main conclusions are independent of the specific mechanism that gives rise to the compact structure.

We analyzed our model by generating ensembles of bacterial DNA configurations with extensive stochastic Monte Carlo computer simulations. A typical result of our model is shown in Figure 1A. As can be seen the model reproduces the experimental results quite well (possible causes of the small differences close to the ori pole are discussed in ref. 12). Similar results were found for newborn E. coli cells. However, here the DNA configurations depend on growth stage and in particular upon initiation of replication different arrangements are found, for details see reference 12. In all cases, the theoretical results do not require a fine-tuning of the parameters but rather are very robust for a large range of parameter values. In particular:

1. The correlation between spatial and genome localization is nearly perfectly linear for sufficiently large blob diameters (i.e., \( d_b \geq 24 \text{ nm} \) for C. crescentus and \( d_b \geq 75 \text{ nm} \) for E. coli).

2. The linear correlation also holds for a large range of blob numbers: for C. crescentus for 200 to 2,000 blobs, for E. coli for 200 to 600 blobs.

3. Linear DNA configurations are also found in a large range of cell volumes. This is important for E. coli that shows a ~10-fold change in volume with growth rate.
(4) With increasing DNA content the linear arrangement of the chromosome becomes stronger. Furthermore, the geometrical ordering also works for a large range of chromosome lengths (ranging from $L = 1.5$ mm to 3 mm) indicating that our proposed mechanism is applicable to different bacteria.

There are also more general conclusions that can be drawn from our analysis. The spatial chromosomal arrangement is quite robust with respect to variations in the positioning of ori and ter. In fact, linear configurations are also found if only ori has a fixed position. In this case ter is free to move but the remaining parts of the chromosome confine its spatial position in this way effectively fixing its position. This implies, that even though ter appears in vivo at a specific position one cannot conclude that this position is fixed by, e.g., anchoring to the pole. Interestingly, an anchoring mechanism for ter has so far not been identified (while it is known that ori is anchored to the flagellated pole by PopZ\textsuperscript{17,18}).

A model that includes only self-avoidance (that, for example, could be induced by electrostatic repulsion between the DNA) but not a mechanism that effectively compacts the chromosome cannot explain the linear correlation. Thus, (sufficiently strong) compaction is essential. This effect cannot be due to supercoiling alone making the action of compaction proteins the most plausible scenario. The importance of compaction was recently also demonstrated in other approaches.\textsuperscript{1,19}

DNA organization is a stochastic process that leads to cell-to-cell variations in the chromosomal arrangement. The linear configuration shown in Figure 1A corresponds to the average configuration of the population. Individual cells can have realizations that deviate quite significantly from this population mean (as indicated by the large standard deviations from the mean curve in Fig. 1A). In fact, individual cells can even have asymmetric DNA configurations, where, e.g., the left strand is closer to the ter pole and the right strand is closer to the ori pole, see Figure 1B. The opposite configuration (with the left [right] strand closer to the ori [ter] pole) occurs with the same probability so that in the population the average configuration is perfectly symmetric. The strength of the symmetry breaking in individual cells can be quantified with the following order parameter

\[
\xi = \frac{|I_1 - I_2|}{|I_1 + I_2|} \quad \text{with} \quad I_1 = \sum_{\text{left strand}} \varepsilon_j \quad \text{and} \quad I_2 = \sum_{\text{right strand}} \varepsilon_j
\]

In the last two equations $\varepsilon_j$ denotes the height of blob $j$ belonging to the left respectively to the right chromosomal strand. The time-average of this quantity is shown in Figure 1C as function of chromosomal length $L$ for different cell volumes $H \times H \times H$. Interestingly, all points calculated from Equation 1 for the different combinations of $L$ and $H$ collapse onto a single curve if plotted as function of $L/H^2$. As one can see symmetry breaking is stronger for longer chromosomes or smaller cells. If the linear organization of the chromosome has a physiological role then one expects that additional mechanisms (such as anchoring of more chromosomal loci) are required to suppress these asymmetric configurations.

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