New and Notable

Chromosome, Cell Cycle, and Entropy

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Look at the beautifully crowded inner space of *Escherichia coli* (Fig. 1; (1)). It is a rich source of inspiration for physicists looking for new problems in biology.

One such problem has been the existence of the nucleoid, a distinct object inside a bacterial cell resulting from the compaction of the chromosomal DNA. That is, fluorescently stained DNA in vivo never fills the entire inner space of *E. coli* (Fig. 1, *inset*), although bacteria do not have an organelle that encapsulates their chromosomes. The measured size of the in vivo nucleoid is not what simple physics would predict. The contour length of the 4.6 Mb single circular *E. coli* chromosome is ~1.5 mm. If the *E. coli* chromosome was a simple random walk of DNA, with the 50 nm = 150 bp persistence length of dsDNA, its natural size would be 50 nm $\times$ $(4.6 \times 10^6/150)^{1/2}$ ~9 μm; approximately one order-of-magnitude larger than the ~1 μm size of a typical *E. coli* cell.

There are two main complementary, but not mutually exclusive, views on the physical origins of the phase separation between the nucleoid and the cytoplasm. Both involve interactions between DNA and proteins, albeit of very different physical natures. For example, there are proteins whose primary role is to condense DNA. Notable examples include histones and condensins in eukaryotes. Bacteria have related proteins collectively known as nucleoid-associated proteins (2). However, overexpression of most nucleoid-associated proteins does not result in further compaction of the nucleoid and, unlike in eukaryotes, their role in compaction of bacterial chromosomes in vivo is not conclusive.

The other view considers the physical consequences of high concentrations of macromolecules in the bacterial cytoplasm. *E. coli* cells growing in nutrient-rich medium can have on the order of 1,000,000 proteins, with intermolecular distances comparable to their own size. Such a high concentration of macromolecules inside a living cell can result in nontrivial physical effects collectively known as "macromolecular crowding".

An important consequence of molecular crowding is the depletion force (3) between otherwise repelling objects, e.g., long DNA strands. In theory, this can cause collapse of chromosomal DNA and thus a phase-separation between the DNA and the proteins inside the cell.

In an important article, Theo Odjik (4) theoretically formalized how macromolecular crowding can collapse supercoiled DNA, and applied the resulting picture to the *E. coli* chromosome. This was further elaborated in an experimental work with Cunha et al. (5), which concluded that the size of the nucleoid decreases continuously as the concentration of the surrounding macromolecules increases.

In stark contrast with this, the transition appeared to be abrupt in more recent experimental results by Pelletier et al. (6). They isolated *E. coli* chromosomes directly in microchannels and observed compaction-decompaction cycles in real-time by periodically injecting and removing varying concentrations of PEGs to mimic a cytoplasmic environment. They showed that around the transition concentration both compact and non-compact nucleoids coexist. This is a signature of a first-order transition. However, when they modified Odjik's theory and used the experimentally measured parameters for the chromosome, their conclusion was "the predicted size of the entropic spring (chromosome) decreases rapidly, albeit continuously…" (6).

If both phenomenological theories (4–6) predict a continuous transition, why does the in vitro chromosome experiment suggest otherwise? This raises an important question: Are depletion forces by molecular crowding sufficient to explain chromosomal collapse in vivo?

A gratifying conclusion is emerging with the work published in this issue of the *Biophysical Journal* by Shendruk et al. (7), who tied together both theoretical and experimental conclusions. They performed coarse-grained molecular dynamics simulations of a model chromosome immersed in a solution of proteins. At first, the article reads like typical polymer physics literature. Their simulated chromosomes are linear chains of spherical monomers, which do not look anything like the long, intricate network of DNA illustrated in Fig. 1. Their cytoplasmic proteins are truncated Lennard-Jones spheres. However, one would be mistaken to dismiss the work by Shendruk et al. (7), because they provide a compelling example of the importance of testing a null hypothesis in biology with physical rigor.

1. Shendruk et al. (7) investigated the size (radius of gyration) of simulated chromosomes in the presence of explicit depletants (i.e., crowding proteins) with a volume fraction up to $\varphi_{dep} = 0.45$, and the number of depletants up to 3×10^5. Such explicit coarse-grained models are computationally very demanding and, if performed well (as is the case in Shendruk et al. (7)), the obtained results are transparent and as valuable as fine data from well-controlled experiments.

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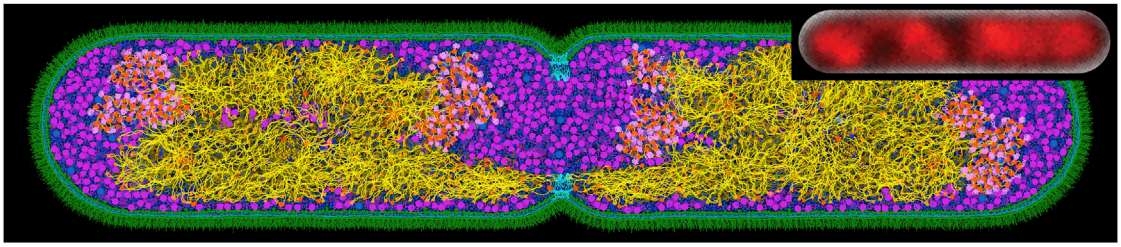

FIGURE 1 Illustration by David Goodsell depicting the interior of *E. coli* during multifork replication under fast growth conditions based on Youngren et al. (1). The illustration includes supercoiled DNA during replication (*yellow*), nucleoid-associated proteins (varying shades of *amber*), cytoplasm (*blue*), ribosomes (*purple*), RNA polymerases (*dark orange*), and RNA (*pink*). Two overlapping cell cycles are taking place with each daughter cell containing two origins of replication. The multifork *E. coli* chromosome is like a branched donut. (*Inset*, *top right*) *E. coli* chromosomes during multifork replication, fluorescently labeled with functional HU-mCherry (6).

2. They use a simple Flory-type theory to analyze the simulation results. As the size ratio between the simulated chromosomal monomers and the depletants was increased, the agreement between the predictions of the theory and the simulations became very good. This allowed the authors to check the order of the compaction-decompaction transition for the parameter space relevant in vivo. For a size ratio between the chromosomal structural unit and the proteins of 20, which is a realistic number (~100 nm chromosomal structural units (8) and a few nanometers for cytoplasmic proteins), their calculations showed a continuous transition.
3. The results of Shendruk et al. (7) show unambiguously that entropic forces by macromolecular crowding are sufficient to cause compaction of chromosomes at the in vivo volume fraction of the proteins. For example, with a size ratio of 20 as stated above, the predicted volume fraction for transition was $\varphi_{dep}^* = 0.03$, whereas the estimated volume fraction of the proteins in vivo is significantly higher, ~0.1–0.2 (6). However, entropic forces alone were not able to produce the first-order transition experimentally observed by Pelletier et al. (6). Thus, they drew a physically sound and biologically important conclusion: nucleoid-associated proteins are the likely explanation for the experimentally observed first-order transition in nucleoid compaction.

This last point is particularly important in interpreting long-standing observations of nucleoid compaction in vivo. It suggests that macromolecular crowding does most of the basal work for nucleoid compaction, and various nucleoid-associated proteins can finish the job to generate a sharp phase separation between the cytoplasm and the nucleoid.

From a more general point of view, the importance of the approach taken in Shendruk et al. (7) is that it establishes a physical guiding principle for interpreting biological phenomena, and tells us what is, and is not, possible based on physical principles.

It is also the beginning of exciting future research on theoretical and experimental fronts. An obvious next step would be to include key biological components such as the cell walls (as in Mondal et al. (9)) to study the effect of spatial confinement of varying confining geometry. Other important factors to consider, while quantitatively assessing their relative importance, include the chromosome topology induced by supercoiling specific to the growth physiology and the cell cycle (Fig. 1) (1). Again, it would be important to focus on establishing the physical basis to help researchers interpret the observed phenomena. These physical guidelines will be most powerful when combined with single-molecule biophysical/biochemical studies, e.g., Wiggins et al. (10), and then more ambitiously, directly tested in vivo.

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