

## MicroCommentary

# A physical approach to segregation and folding of the *Caulobacter crescentus* genome

Remus T. Dame,<sup>1,2\*</sup> Mariliis Tark-Dame<sup>3</sup> and Helmut Schiessel<sup>4</sup>

<sup>1</sup>Leiden Institute of Chemistry, Gorlaeus Laboratories, Laboratory of Molecular Genetics and Cell Observatory, Leiden University, Leiden, the Netherlands.

<sup>2</sup>Department of Physics and Astronomy, VU University Amsterdam, Amsterdam, the Netherlands.

<sup>3</sup>Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands.

<sup>4</sup>Instituut Lorentz, Leiden University, Leiden, the Netherlands.

### Summary

**Bacterial genomes are functionally organized. This organization is dynamic and globally changing throughout the cell cycle. Upon initiation of replication of the chromosome, the two origins segregate and move towards their new location taking along the newly replicated genome. *Caulobacter crescentus* employs a dedicated active partitioning (Par) system to move one copy of the *parS* centromere to the distal pole, while the other stays at the stalked pole. In this issue of *Molecular Microbiology*, Hong and McAdams describe studies on the speed of segregation of *parS* and regions up to 150 kb away. They show clear differences in segregation rates between *parS* and 50 kb flanking regions versus regions further away. To assess segregation rates the authors track fluorescent markers during movement using time-lapse microscopy. The relation between genomic and physical distance of pairs of markers reflects how the genome is folded. This relation permits testing experimental data against models from polymer physics. Such models are helpful in understanding principles of genome folding. Although long used in studies on eukaryotes, this approach has rarely been applied to bacteria. Finally, the authors give the first direct**

**evidence for a role of the bacterial chromatin protein HU in folding the genome *in vivo*.**

Understanding the spatial and functional organization of bacterial genomes throughout the cell cycle is important to understanding their physiology. It is fascinating to learn what mechanisms have evolved to organize the genome and reduce its effective volume below that of a cell (Dame, 2005; Luijsterburg *et al.*, 2008; Dillon and Dorman, 2010; Dame *et al.*, 2011). The organization of the bacterial genome is hierarchical, occurring at different length scales. On the micrometer scale it is organized in macrodomains (Niki *et al.*, 2000; Boccard *et al.*, 2005). In *Escherichia coli* four such domains, each of them on the order of 1 Mbp in size, have been described: the *Ori* and *Ter* domains and the *Left* and *Right* domains flanking *Ter*. Although, it is poorly understood how these domains are organized and what demarcates their borders, recent studies have demonstrated that certain architectural proteins are exclusively associated to certain domains (Mercier *et al.*, 2008; Sanchez-Romero *et al.*, 2010; Cho *et al.*, 2011; Tonthat *et al.*, 2011). Within macrodomains at intermediate scales the genome is organized in topologically independent domains (loops) of an average size of 10 kbp (Postow *et al.*, 2004; Deng *et al.*, 2005; Noom *et al.*, 2007). Also in this case it is not well understood how these loops are formed, but it has been suggested that they arise by clustering of DNA-bound proteins, such as RNA polymerase [in transcription factories (Cook, 2010)] or architectural proteins that bind and bridge sites or regions that are distant along the genome, such as H-NS and SMC/MukBEF (Dame *et al.*, 2000; 2006; Petrushenko *et al.*, 2010; Schwartz and Shapiro, 2011). Finally, there are numerous architectural proteins that shape the path of the genome on the nanometre scale by their ability to bridge or bend the DNA at their binding site. In *E. coli* this includes proteins such as H-NS, FIS, HU and IHF (Luijsterburg *et al.*, 2008; Dillon and Dorman, 2010). Although not all of the relevant architectural proteins identified in *E. coli* are conserved in *Caulobacter crescentus*, it is reasonable to assume that similar general principles of genome organization apply also to this organism.

Organization and remodelling of the genome throughout the cell cycle and in response to environmental cues are key determinants in tuning the physiological state of bacteria.

Upon replication of the *C. crescentus* genome active transport of the *ori* (followed by the rest of the newly replicated genome) towards the distal pole occurs. This transport is facilitated by interaction of the *parS* site adjacent to the *ori* with the ParB protein, which in turn associates with ParA. ParA is present as polymerized structure extending from the distal pole and it is believed that depolymerization of this structure is the driving force to pull the newly replicated chromosome via its *parS* site to the distal pole (Ptacin *et al.*, 2010; Schofield *et al.*, 2010; Shebelut *et al.*, 2010; Banigan *et al.*, 2011). Previous studies have demonstrated that the speed by which the two *parS* sites separate is not constant throughout the segregation phase (Viollier *et al.*, 2004; Shebelut *et al.*, 2010). In the current study, Hong and McAdams have measured the separation velocity of the *parS* sites and sites up to ~150 kb away from *parS*. The authors used time-lapse fluorescent microscopy to track the subcellular positions of these sites during chromosome segregation, which permits calculation of translocation speeds. Their experiments show that a site proximal to *parS* and sites that are further away exhibit different behaviour. Loci ~100 and 150 kb away separate at a much higher speed than the *parS* sites. The site at ~50 kb exhibits a mixed segregation pattern behaving like the *parS* site in one-third of the observed cells and separating at the same velocity as the more distant loci in the remaining two-thirds of the cells. Loci at ~10 kb distance generally exhibit only slow segregation, identical to that of *parS*. For comparison, it takes the *parS* site 5 min to reach the distal pole, whereas transport of distant loci is completed in about half a minute. The authors suggest that these differences are due to the existence of two segregation mechanisms. They propose that in addition to the slow segregation mediated by the Par system, a second – currently undefined – mechanism is operating that facilitates fast segregation. An alternative explanation is that these differences can be attributed to the build-up of tension during transport that might relate to differences in structural organization of the regions nearby *parS* (here defined as the region up to the first locus at 12 kb) and those further away. In fact, the locus at 12 kb in part of the cells follows the *parS* site at the same slow speed, whereas in other cells it follows after a time delay at high speed. Loci further away generally segregate at high speed (up to 2.5 times the speed of the *parS* site). It is unclear where this heterogeneity in behaviour of the *parS* proximal region derives from, but at least in a fraction of cells this observation can be interpreted as this region being less sensitive to stretching by force than distal

regions. This could imply the existence of a stable compact region (characterized by a high stiffness) close to *parS* and distal regions that are more easily extended by the application of force. When reaching a critical tension this 'extended spring' relaxes and the DNA can be transported to the distant pole. The authors give qualitative insight into the response to force of the region up to 12 kb away from *parS*. The distance from the 12 kb locus to *parS* increases as a function of *parS* speed (a proxy for the applied force). As similar data are lacking for longer distances our predictions relating to the different stiffness of the two regions can unfortunately not be verified.

An interesting question is: what delays the transport of these distant loci? An obvious candidate to delay transport is the replisome and the rate of replication. A back of the envelope calculation shows that this is indeed a possibility. Replication proceeds at a rate of on average about 21 kbp min<sup>-1</sup> (Dingwall and Shapiro, 1989), while the speed of *parS* movement measured by Hong and McAdams is in the order of 1–3 μm min<sup>-1</sup>, i.e. approximately 3–10 kbp min<sup>-1</sup> assuming that the DNA is fully extended during segregation. However, this is not the case as the genome is compacted (see next paragraph). As the authors show, for instance a region of 20 kb of DNA in the *parS* proximal region has an average end-to-end distance of 0.3 μm.

In addition to segregation speed, Hong and McAdams investigated the folding of an approximately 30 kb genomic region directly adjacent to *parS* by measuring the mean physical distance  $R$  between *parS* and ten different loci up to 33 kbp away. They found a scaling law of the form  $R \propto g^{0.34}$  with  $g$  denoting the genomic distance between measured loci. The relation between the physical and the genomic distance can be compared with polymer models to estimate the folding state of the region of interest. This approach has not yet been applied to studies in bacteria but has been widely used in the field of eukaryotic chromatin during the last one and a half decade (Emanuel *et al.*, 2009; Tark-Dame *et al.*, 2011). A hallmark of polymer models is that the mean distance between pairs of monomers,  $R$ , exhibits scaling laws of the form  $R \propto g^{\nu}$  where  $g$  denotes the distance between the monomers along the chain.  $\nu$  is a scaling exponent that takes different values depending on the chain conformation. For a self-avoiding chain one finds  $\nu \approx 3/5$  whereas for a situation where the excluded volume can be neglected, one finds the ideal chain exponent  $\nu = 1/2$ . Both exponents are larger than 0.34, the value observed by Hong and McAdams. That value seems to suggest a compact object for which the overall size scales like the number of building blocks to the power 1/3. In fact, the size of a polymer globule in a poor solvent scales like  $N^{1/3}$  with  $N$  denoting the total number of monomers. Remarkably, however, this law does not apply to intermonomer

distances inside the globule. In fact, screening of the excluded volume due to the presence of other monomers leads to an exponent 1/2 (Lua *et al.*, 2004). All the standard polymer models thus show larger exponents than 0.34.

How can the small value of the exponent be explained? To start with, let us stress the remarkable fact that a similar value of that exponent, approximately 1/3, has been derived for eukaryotic DNA organization from the contact probability between pairs of loci between ~ 500 kb and ~ 7 Mb (Lieberman-Aiden *et al.*, 2009; Mirny, 2011). The authors of that paper suggested that this might reflect the organization of eukaryotic chromatin in the form of a fractal globule, an object as compact as the ordinary globule discussed above but in addition self-similar on all length scales as each of its subchains is condensed in itself. A remarkable feature of such structures is that they have very few topological entanglements. This might be advantageous from a biological point of view since it allows large-scale opening and closing of sections of the genome. Based on that idea the fractal (or crumpled) globule had already been suggested long ago on purely theoretical grounds as a possible candidate for eukaryotic DNA organization (Grosberg *et al.*, 1988; 1993; Halverson *et al.*, 2011; Mirny, 2011). It should be mentioned, however, that unlike the highly entangled standard globules, this is a non-equilibrium structure. Such structures might be possible due to the huge lengths of eukaryotic DNA that would need hundreds of years to reach equilibrium in the absence of enzymes involved in disentanglement (e.g. topoisomerases) (Rosa and Everaers, 2008). It should be noted that in addition to the fractal globule model there are several other non-standard polymer models explaining chromatin folding (Emanuel *et al.*, 2009; Tark-Dame *et al.*, 2011).

For the much shorter bacterial genomes where relaxation times are on the order of a few minutes, it seems unlikely that the DNA shows a non-equilibrium conformation. How can then the small value of the scaling exponent be explained in this case? Instead the small value might reflect genomic conformations resembling the ones of branched or looped polymers. Both types of polymers are much more compact than linear polymers (Cunha *et al.*, 2001; Bohn *et al.*, 2007). Indeed, as mentioned by Hong and McAdams, supercoiled DNA resembles branched polymers. This might thus explain the small exponent found in their experiment. Consider a branched polymer with  $N$  monomers where a fraction  $f$  of the monomers leads to multifunctional branches. A path spanning the whole structure has  $m < N$  monomers from which  $fm$  arms, each on average  $m$  monomers long, branch off; hence  $m \propto N^{1/2}$ . For an ideal chain this path forms a random walk leading to an overall size of the branched polymer proportional to  $N^{1/4}$ ; in a good

solvent the path is substantially stretched and the overall size scales like  $N^{1/2}$  (Cunha *et al.*, 2001). The observation by Hong and McAdams of a value of about 1/3 might thus very well reflect a branched structure of the *C. crescentus* genome in the 30 kb large region directly adjacent to *parS*. Since it is not straightforward to unambiguously assign a certain type of folding based purely on the value of the exponent, more data will be necessary to come to a definite conclusion about this point.

Interestingly, earlier measurements by Shapiro and coworkers on longer distances (up to 2 Mbp, the distance between *ori* and *ter*), have reported linear organization of the *C. crescentus* genome between *ori* and *ter*, i.e. the physical distance scales linearly with genomic distance (Viollier *et al.*, 2004). Although the data by Hong and McAdams on the much shorter region relate to a period of active transport, it implies different types of organization at different length scales. An illuminating recent study on the overall three-dimensional organization of the *C. crescentus* genome relying on a Chromosome Conformation Capture-based approach (Dekker *et al.*, 2002) by Church and coworkers (Umbarger *et al.*, 2011) revealed that the chromosome is folded as an ellipsoid, with the arms between *ori* and *ter* spiralling around each other. To date that approach, however, does not have the resolution to provide information on the folding at short-length scales, the scale at which architectural proteins are likely to act and that is investigated here.

Studies in which the *in vivo* contribution of an architectural protein to folding of a genomic region is evaluated using polymer physics models have not been reported to date for any organism. As the authors focused their studies on short-length scales they were in an ideal position to investigate the role of a bacterial architectural protein *in vivo*. HU was one of the first proteins proposed to have an architectural role in the folding of the bacterial genome (Rouviere-Yaniv and Gros, 1975). The protein is present in several tens of thousands copies per cell, it binds DNA with low sequence specificity, is associated with the folded bacterial genome *in vivo* (Shellman and Pettijohn, 1991; Wery *et al.*, 2001; Lee *et al.*, 2011; Wang *et al.*, 2011) and has been shown to compact DNA *in vitro* (Skoko *et al.*, 2004; van Noort *et al.*, 2004). An effect of HU on genome folding has however not been directly demonstrated *in vivo*. The role of the two *C. crescentus* HU proteins was investigated here by assessing their effect on the folding of 30 kb regions on both sides of *parS* using single and double knockout strains. The authors convincingly show that the measured distance between loci along the genome and the *parS* site is larger in the HU-1, HU-2 double knockout strain than in the wild type. Similarly, at all but one of the probed loci deletion of HU-2 results in decompaction of the genome. Deletion of HU-1 does not result in any measurable change in compaction.

This observation may at first sight seem surprising, but might be accounted for by *in vitro* data on the *E. coli* HU proteins, demonstrating that one of the HU homodimers has much lower DNA binding affinity than the other type of homodimer or the heterodimer (Pinson *et al.*, 1999).

The availability of techniques that permit assessing genome folding in the cell has been driving the progress in understanding chromatin organization and dynamics in eukaryotic organisms. The type of data generated by quantitative Chromosome Conformation Capture and imaging techniques is very well suited to interpretation using polymer physics models (Emanuel *et al.*, 2009; Mirny, 2011; Tark-Dame *et al.*, 2011). However, it is hard to pinpoint exactly which model best suits the data as the value of the scaling exponent alone does not permit conclusive assignment of the 'best' polymer model. The bacterial chromatin organization field is now catching up, providing the first glimpses into local (Hong and McAdams, 2011) as well as global genome folding (Viollier *et al.*, 2004; Wiggins *et al.*, 2010; Umbarger *et al.*, 2011). It will be very insightful to assess and validate the roles of other potential chromatin organizing proteins in *C. crescentus* and to apply and extend these approaches to other bacterial model organisms.

## Acknowledgements

Research in the authors' laboratories is financially supported through a VIDJ grant from the Netherlands Organization for Scientific Research (grant 864.08.001 to R.T.D.), the NanoN-extNL '8B: Bionano interactions for biosensing' programme (R.T.D.) and the 'DNA in action: Physics of the Genome' programme of the Foundation for Fundamental Research on Matter (grant FOMA29-07DNAA11 to M.T.D. and grant FOML26-07DNAA07 to H.S.).

## References

- Banigan, E.J., Gelbart, M.A., Gitai, Z., Wingreen, N.S., and Liu, A.J. (2011) Filament depolymerization can explain chromosome pulling during bacterial mitosis. *PLoS Comput Biol* **7**: e1002145.
- Boccard, F., Esnault, E., and Valens, M. (2005) Spatial arrangement and macrodomain organization of bacterial chromosomes. *Mol Microbiol* **57**: 9–16.
- Bohn, M., Heermann, D.W., and van Driel, R. (2007) Random loop model for long polymers. *Phys Rev E Stat Nonlin Soft Matter Phys* **76**: 051805.
- Cho, H., McManus, H.R., Dove, S.L., and Bernhardt, T.G. (2011) Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist. *Proc Natl Acad Sci USA* **108**: 3773–3778.
- Cook, P.R. (2010) A model for all genomes: the role of transcription factories. *J Mol Biol* **395**: 1–10.
- Cunha, S., Woldringh, C.L., and Odijk, T. (2001) Polymer-mediated compaction and internal dynamics of isolated *Escherichia coli* nucleoids. *J Struct Biol* **136**: 53–66.
- Dame, R.T. (2005) The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin. *Mol Microbiol* **56**: 858–870.
- Dame, R.T., Wyman, C., and Goosen, N. (2000) H-NS mediated compaction of DNA visualised by atomic force microscopy. *Nucleic Acids Res* **28**: 3504–3510.
- Dame, R.T., Noom, M.C., and Wuite, G.J. (2006) Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation. *Nature* **444**: 387–390.
- Dame, R.T., Kalmykova, O.J., and Grainger, D.C. (2011) Chromosomal macrodomains and associated proteins: implications for DNA organization and replication in gram negative bacteria. *PLoS Genet* **7**: e1002123.
- Dekker, J., Rippe, K., Dekker, M., and Kleckner, N. (2002) Capturing chromosome conformation. *Science* **295**: 1306–1311.
- Deng, S., Stein, R.A., and Higgins, N.P. (2005) Organization of supercoil domains and their reorganization by transcription. *Mol Microbiol* **57**: 1511–1521.
- Dillon, S.C., and Dorman, C.J. (2010) Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat Rev Microbiol* **8**: 185–195.
- Dingwall, A., and Shapiro, L. (1989) Rate, origin, and bidirectionality of *Caulobacter* chromosome replication as determined by pulsed-field gel electrophoresis. *Proc Natl Acad Sci USA* **86**: 119–123.
- Emanuel, M., Radja, N.H., Henriksson, A., and Schiessel, H. (2009) The physics behind the larger scale organization of DNA in eukaryotes. *Phys Biol* **6**: 025008.
- Grosberg, A.Y., Nechaev, S.K., and Shakhnovich, E.I. (1988) The role of topological constraints in the kinetics of collapse of macromolecules. *J Phys France* **49**: 2095–2100.
- Grosberg, A.Y., Rabin, Y., Havlin, S., and Neer, A. (1993) Crumpled globule model of the three-dimensional structure of DNA. *Europhys Lett* **23**: 373–378.
- Halverson, J.D., Lee, W.B., Grest, G.S., Grosberg, A.Y., and Kremer, K. (2011) Molecular dynamics simulation study of nonconcatenated ring polymers in a melt. I. Statics. *J Chem Phys* **134**: 204904.
- Hong, S.H., and McAdams, H.H. (2011) Compaction and transport properties of newly replicated *Caulobacter crescentus* DNA. *Mol Microbiol* **82**: 1349–1358.
- Lee, S.F., Thompson, M.A., Schwartz, M.A., Shapiro, L., and Moerner, W.E. (2011) Super-resolution imaging of the nucleoid-associated protein HU in *Caulobacter crescentus*. *Biophys J* **100**: L31–L33.
- Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., *et al.* (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**: 289–293.
- Lua, R.C., Borovinskiy, A.L., and Grosberg, A.Y. (2004) Fractal and statistical properties of large compact polymers: a computational study. *Polymer* **45**: 717–731.
- Luijsterburg, M.S., White, M.F., van Driel, R., and Dame, R.T. (2008) The major architects of chromatin: architectural proteins in bacteria, archaea and eukaryotes. *Crit Rev Biochem Mol Biol* **43**: 393–418.
- Mercier, R., Petit, M.A., Schbath, S., Robin, S., El Karoui, M., Boccard, F., and Espeli, O. (2008) The MatP/matS site-

- specific system organizes the terminus region of the *E. coli* chromosome into a macrodomain. *Cell* **135**: 475–485.
- Mirny, L.A. (2011) The fractal globule as a model of chromatin architecture in the cell. *Chromosome Res* **19**: 37–51.
- Niki, H., Yamaichi, Y., and Hiraga, S. (2000) Dynamic organization of chromosomal DNA in *Escherichia coli*. *Genes Dev* **14**: 212–223.
- Noom, M.C., Navarre, W.W., Oshima, T., Wuite, G.J., and Dame, R.T. (2007) H-NS promotes looped domain formation in the bacterial chromosome. *Curr Biol* **17**: R913–R914.
- van Noort, J., Verbrugge, S., Goosen, N., Dekker, C., and Dame, R.T. (2004) Dual architectural roles of HU: formation of flexible hinges and rigid filaments. *Proc Natl Acad Sci USA* **101**: 6969–6974.
- Petrushenko, Z.M., Cui, Y., She, W., and Rybenkov, V.V. (2010) Mechanics of DNA bridging by bacterial condensin MukBEF *in vitro* and *in singulo*. *EMBO J* **29**: 1126–1135.
- Pinson, V., Takahashi, M., and Rouviere-Yaniv, J. (1999) Differential binding of the *Escherichia coli* HU, homodimeric forms and heterodimeric form to linear, gapped and cruciform DNA. *J Mol Biol* **287**: 485–497.
- Postow, L., Hardy, C.D., Arsuaga, J., and Cozzarelli, N.R. (2004) Topological domain structure of the *Escherichia coli* chromosome. *Genes Dev* **18**: 1766–1779.
- Ptacin, J.L., Lee, S.F., Garner, E.C., Toro, E., Eckart, M., Comolli, L.R., *et al.* (2010) A spindle-like apparatus guides bacterial chromosome segregation. *Nat Cell Biol* **12**: 791–798.
- Rosa, A., and Everaers, R. (2008) Structure and dynamics of interphase chromosomes. *PLoS Comput Biol* **4**: e1000153.
- Rouviere-Yaniv, J., and Gros, F. (1975) Characterization of a novel, low-molecular-weight DNA-binding protein from *Escherichia coli*. *Proc Natl Acad Sci USA* **72**: 3428–3432.
- Sanchez-Romero, M.A., Busby, S.J., Dyer, N.P., Ott, S., Millard, A.D., and Grainger, D.C. (2010) Dynamic distribution of seqA protein across the chromosome of *Escherichia coli* K-12. *MBio* **1**: 00012-10.
- Schofield, W.B., Lim, H.C., and Jacobs-Wagner, C. (2010) Cell cycle coordination and regulation of bacterial chromosome segregation dynamics by polarly localized proteins. *EMBO J* **29**: 3068–3081.
- Schwartz, M.A., and Shapiro, L. (2011) An SMC ATPase mutant disrupts chromosome segregation in *Caulobacter*. *Mol Microbiol* **82**: 1359–1374.
- Shebelut, C.W., Guberman, J.M., van Teeffelen, S., Yakhnina, A.A., and Gitai, Z. (2010) *Caulobacter* chromosome segregation is an ordered multistep process. *Proc Natl Acad Sci USA* **107**: 14194–14198.
- Shellman, V.L., and Pettijohn, D.E. (1991) Introduction of proteins into living bacterial cells: distribution of labeled HU protein in *Escherichia coli*. *J Bacteriol* **173**: 3047–3059.
- Skoko, D., Wong, B., Johnson, R.C., and Marko, J.F. (2004) Micromechanical analysis of the binding of DNA-bending proteins HMGB1, NHP6A, and HU reveals their ability to form highly stable DNA-protein complexes. *Biochemistry* **43**: 13867–13874.
- Tark-Dame, M., van Driel, R., and Heermann, D.W. (2011) Chromatin folding – from biology to polymer models and back. *J Cell Sci* **124**: 839–845.
- Tonthat, N.K., Arold, S.T., Pickering, B.F., Van Dyke, M.W., Liang, S., Lu, Y., *et al.* (2011) Molecular mechanism by which the nucleoid occlusion factor, SlmA, keeps cytokinesis in check. *EMBO J* **30**: 154–164.
- Umbarger, M.A., Toro, E., Wright, M.A., Porreca, G.J., Bau, D., Hong, S.H., *et al.* (2011) The three-dimensional architecture of a bacterial genome. *Mol Cell* **44**: 252–264.
- Viollier, P.H., Thanbichler, M., McGrath, P.T., West, L., Meewan, M., McAdams, H.H., and Shapiro, L. (2004) Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc Natl Acad Sci USA* **101**: 9257–9262.
- Wang, W., Li, G.W., Chen, C., Xie, X.S., and Zhuang, X. (2011) Chromosome organization by a nucleoid-associated protein in live bacteria. *Science* **333**: 1445–1449.
- Wery, M., Woldringh, C.L., and Rouviere-Yaniv, J. (2001) HU-GFP and DAPI co-localize on the *Escherichia coli* nucleoid. *Biochimie* **83**: 193–200.
- Wiggins, P.A., Cheveralls, K.C., Martin, J.S., Lintner, R., and Kondev, J. (2010) Strong intranucleoid interactions organize the *Escherichia coli* chromosome into a nucleoid filament. *Proc Natl Acad Sci USA* **107**: 4991–4995.