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Macromolecular crowding can account for RNase-sensitive constraint of bacterial nucleoid structure

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ABSTRACT

The shape and compaction of the bacterial nucleoid may affect the accessibility of genetic material to the transcriptional machinery in natural and synthetic systems. To investigate this phenomenon, the nature and contribution of RNA and protein to the compaction of nucleoids that had been gently released from *Escherichia coli* cells were investigated using fluorescent and transmission electron microscopy. We propose that the removal of RNA from the bacterial nucleoid affects nucleoid compaction by altering the branching density and molecular weight of the nucleoid. We show that a common detergent in nucleoid preparations, Brij 58, plays a previously unrecognized role as a macromolecular crowding agent. RNA-free nucleoids adopt a compact structure similar in size to exponential-phase nucleoids when the concentration of Brij 58 is increased, consistent with our hypothesis. We present evidence that control and protein-free nucleoids behave similarly in solutions containing a macromolecular crowding agent. These results show that the contribution to DNA compaction by nucleoid-associated proteins is small when compared to macromolecular crowding effects.

1. Introduction

In vivo, the bacterial nucleoid is compacted over one thousand fold during the log-growth phase [1], however the majority of the bacterial chromosome exists in a transcriptionally accessible state [2,3]. Given recent advances toward building synthetic genomes *de novo*, we aim to understand whether chromosomal compaction results from physical properties of the biomolecules involved, or if specific molecular mechanisms enable this accessibility and will therefore must be incorporated into synthetic genome design.

Several physical forces contribute to bacterial nucleoid compaction. Chromosomal DNA is negatively supercoiled through the action of several topoisomerases, and cellular polyamines such as spermidine and spermine are thought to participate in DNA compaction by partially neutralizing the charge of the DNA backbone [4]. *In vitro*, physiological levels of polyamines compact DNA several fold by inducing a first order random coil to globule transition for naked DNA chains [5–8]. Cytoplasmic macromolecular crowding also plays a role in chromosomal compaction. The concentration of macromolecules (proteins and RNA) in bacterial cytoplasm is approximately 340 mg/mL, producing significant compaction forces due to excluded volume effects [1,9–11]. Compaction of DNA by macromolecular crowding has been observed *in vitro* as a model for *in vivo* processes [7,12,13], but the extent of its contribution *in vivo* is still ambiguous.

In addition to physical forces, the nucleoid may be compacted by biochemical forces, namely RNA and nucleoid-associated proteins (NAPs). An RNA-based DNA compaction force has been observed for isolated nucleoids [1,14–16]; yet the nature of an RNA constraint remains controversial. The removal of RNA from nucleoid preparations causes nucleoid instability and decompaction regardless of whether the RNA is removed *in vivo* (rifampicin-treated cells) or following nucleoid release (RNase-treated nucleoids). However, the RNA molecule(s) responsible for nucleoid stabilization has not been identified. Similarly, several NAPs exist in bacteria, many of which can compact DNA *in vitro* [17]. However this function has been called into question. NAP-mediated DNA compaction *in vitro* requires protein levels that are several times higher than intracellular levels. In addition, genomic deletion of specific NAPs does not appear to alter global DNA structure [18]. Zimmerman has argued that NAPs may not compact DNA *in vivo*, since removing NAPs by washing has little effect on the size of isolated nucleoids [12].

Given recent advances toward the design and synthesis of a synthetic genome [19–23], we wanted to determine whether specific proteins or RNA molecules are required to maintain

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functional and accessible genomes in a simplified bacterial cell. We hypothesized that nucleoid compaction is mediated mainly by physical forces, specifically macromolecular crowding effects, and that specific RNA molecules and proteins are not necessary to achieve overall chromosomal compaction in bacteria. Here, we present evidence that previously observed RNA-based DNA compaction forces result from changes to the physical structure of the nucleoid, and that these changes may affect interactions between the nucleoid and surrounding polymers. Specifically, we present evidence that Brij 58, a detergent used extensively in *Escherichia coli* nucleoid preparations, also serves an unrecognized role as a macromolecular crowding agent in these preparations. The removal of RNA from the nucleoid either *in vivo* or following cell lysis resulted in reduced Brij 58-dependent nucleoid compaction. In addition, we present evidence that the contribution of nucleoid-associated proteins to nucleoid compaction is small when compared to macromolecular crowding effects, as native and protein-free nucleoids behaved similarly with respect to changes in macromolecular crowding.

2. Materials and methods

2.1. Bacterial strain and growth conditions

Escherichia coli B/r A (ATCC 12407) was grown in C-medium (17.2 mM dibasic potassium phosphate, 11.0 mM monobasic potassium phosphate, 9.5 mM ammonium sulfate, 0.41 mM magnesium sulfate, 0.17 mM sodium chloride, 3.6 μM ferrous sulfate, 1.0 μM EDTA) containing 0.1% glucose [24] at 37 °C, shaking at 350 rpm to OD_{600} ~0.4. The doubling time for these cultures was 44 min. Where indicated, chloramphenicol or rifampicin was added to a final concentration of 30 μg/mL or 40 μg/mL, respectively, and cultures were incubated at 37 °C, 350 rpm for 30 min. Cells were harvested by centrifugation at 14,000*g*, 4 °C for 10 min.

2.2. Nucleoid preparations for fluorescence microscopy

Nucleoids for fluorescent microscopy were isolated using a modified version of the low-salt procedure developed by Murphy and Zimmerman [10]. Briefly, harvested cell pellets were resuspended in 250 μL Solution A (20% sucrose, 10 mM Tris–HCl, pH 8.1, 0.1 M sodium chloride) containing a 1/200 dilution of Picogreen (Invitrogen Co., Carlsbad, CA) and mixed briefly. After 2 min, 50 μL of Solution B (4 mg lysozyme per mL in 120 mM Tris–HCl, pH 8.1, 50 mM EDTA) were added and suspensions were held at room temperature for 1 min. Ten microliters of the suspension was diluted 10-fold into Solution D (7.1% sucrose, 14 mM Tris–HCl, pH 8.1, 36 mM sodium chloride, 10 mM EDTA, 5 mM spermidine, 5 mg Brij 58/mL, 0.22% sodium deoxycholate). The concentrations of Brij 58 and spermidine were varied as indicated. Cells were incubated at room temperature for >20 min to allow nucleoid release. The final nucleoid solution contained the same concentration of all components except Picogreen as the previously described low-salt procedure. Aliquots of each nucleoid suspension were viewed with an Olympus BX-51 fluorescent microscope and imaged using a Cooke SensiCam CCD camera. The images were analyzed with ImageJ version 1.38x software (NIH, Bethesda MD). The contrast of the images was adjusted to the same level and the area of each nucleoid was measured three times using the freehand selection tool. The average area was taken to be the nucleoid area.

2.3. Fragmented E. coli chromosomal DNA preparations

Escherichia coli B/r cell suspensions (10 mL, OD_{600} ~0.4) were centrifuged for 10 min at 14,000*g*, 4 °C. DNA was isolated from cell pellets using the FastDNA® Kit and the FastPrep® Instrument (Qbiogene, Inc., CA). The size of the fragmented DNA ranged from 5 to 6.5 kb as measured by agarose gel electrophoresis (data not shown). Fragmented DNA was diluted to 2.5 μg/mL in solutions containing a 1/200 dilution of Picogreen with Brij 58 and sodium chloride or spermidine. DNA compaction was observed by fluorescent microscopy as previously described.

2.4. Nucleoid isolation for electron microscopy

Nucleoids were isolated from harvested *E. coli* B/r A cells using a high- or low-salt procedure. The 'high-salt procedure' developed by Stonington and Pettijohn was employed with the following modifications [2]. Harvested cell pellets were resuspended in 250 μL Solution A and mixed briefly. After 2 min, 50 μL of Solution B was added and the samples were inverted to mix. After 1 min, 250 μL of Solution C (1% Brij 58, 0.4% sodium deoxycholate, 10 mM EDTA, pH 8.0, 2 M sodium chloride) was added and the samples were incubated at room temperature for 10–20 min, until the suspensions began to clear. Nucleoid suspensions were layered onto 10–30% sucrose continuous gradients in 10 mM Tris–HCl, pH 8.1, 1 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and centrifuged at 28,000*g*, 4 °C for 45 min in a Beckman SW41 rotor. Acceleration and deceleration were set to one. The 'low-salt procedure' was based on Kornberg et al.'s modification of the Stonington and Pettijohn procedure, where the 2 M NaCl in Solution C was replaced with 10 mM spermidine [25]. In addition, cell suspensions were incubated at 37 °C for 5 min to allow cell lysis. Nucleoid suspensions from low-salt preparations were loaded onto 15–30% sucrose continuous gradients in 20 mM sodium diethylmalonate, pH 7.1, 5 mM magnesium chloride, 1 mM β-mercaptoethanol. Gradients were centrifuged at 3000*g*, 4 °C for 35 min in a Beckman SW41 rotor. Acceleration and deceleration were set to one.

Gradients from both high- and low-salt preparations were fractionated into 0.3 mL aliquots. Picogreen was added to a portion of each fraction to a final dilution of 1/400, and the DNA concentration of each fraction was determined according to manufacturer's recommendations (Invitrogen Co., Carlsbad, CA).

2.5. Electron microscopy of isolated nucleoids

Nucleoids were attached to Butvar-coated nickel grids that were freshly coated with carbon by glow-discharge as described by Postow et al. [26]. Aliquots (8 μL) from the peak fraction were adsorbed to the grid for 2 min. Grids were rinsed for consecutive 1 min intervals in 0.1 M ammonium acetate, 0.01 M ammonium acetate, and 2% uranyl acetate. Grids were viewed using a Tecnai T12 transmission electron microscope (FEI Co., Hillsboro, Oregon), and imaged with an SIS Megaview III CCD camera. Images were analyzed with ImageJ software. The condensed area of each nucleoid was measured three times using the freehand selection tool, and averaged. Membrane fragments and single loops extending from the central body of the nucleoid were excluded.

3. Results

3.1. Brij 58 serves as macromolecular crowding agent in nucleoid preparations

Escherichia coli nucleoids were prepared using a low-salt procedure in which *E. coli* cells are sequentially exposed to a sucrose/sodium chloride solution to disrupt the outer membrane, lysozyme to degrade the peptidoglycan layer, and finally a stabilizing solution containing Brij 58, sodium deoxycholate, EDTA, and spermidine (final concentrations 1%, 0.4%, 10 mM and 10 mM, respec-

tively) to solubilize the inner membrane and stabilize the compacted nucleoid. To investigate the forces responsible for stabilizing compacted nucleoids, we removed each reagent from the stabilizing solution and observed the effect on nucleoid preparations via fluorescent microscopy. Removal of either EDTA or sodium deoxycholate resulted in incomplete cell lysis (Fig. 1C and D), while the removal of spermidine resulted in partial decompaction of the nucleoids (Fig. 1E). Removal of Brij 58 from the reaction resulted in nearly complete decompaction of the nucleoid DNA (Fig. 1F). Very few intact cells were observed in the nucleoid preparations lacking either spermidine or Brij 58 confirming that cell lysis is not prevented by removal of either component. We concluded that both spermidine and Brij 58 play a role in *in vitro* nucleoid stabilization.

Brij 58 is a non-ionic detergent used to permeabilize the *E. coli* cells during lysis, but our results indicated it also served an unrecognized role as a macromolecular crowding agent that stabilized compacted nucleoids following lysis. To further investigate the role of Brij 58 in DNA compaction, we prepared purified, fragmented *E. coli* chromosomal DNA that lacked proteins and RNA molecules hypothesized to participate in chromosomal compaction, and observed DNA compaction in the presence of Brij 58. Solutions with only Brij 58 required much higher levels than those found in nucleoid preparations to compact naked DNA independently of spermidine (>100 mg/mL Brij 58 versus 5 mg/mL Brij 58). We found the ionic strength of the solution affected Brij 58-mediated compaction of naked DNA; high levels of sodium chloride decreased the critical Brij 58 concentration required for DNA collapse (Fig. 2). These observations agree well with previous studies that characterized the conditions required for compaction of DNA molecules in the presence of macromolecular poly(ethylene glycol) (i.e., PEG) and poly(ethylene oxide) (i.e., PEO) [27–29]. Together with the structural similarities between Brij 58 and PEG (Fig. 2), our results show that Brij 58 participates in nucleoid compaction through a mechanism similar to PEG, presumably through macromolecular crowding effects.

3.2. Compaction of RNA-free nucleoids requires high levels of Brij 58

Based on our observations that Brij 58 serves as a macromolecular crowding factor in nucleoid preparations, we hypothesized that the nucleoid decompaction observed in RNA-free nucleoids may result from changes in the physical structure of the nucleoid that affect Brij 58-induced crowding. We investigated Brij 58-mediated nucleoid compaction in native nucleoids (nucleoids isolated from cells in exponential phase, with no antibiotic, RNase A treatment), and compared the compaction with that of RNA-free nucleoids (prepared either *in vivo* by rifampicin treatment or following cell lysis by RNAse A treatment) (Table 1). Our results show that native nucleoids are compacted with concentrations of Brij 58 greater than or equal to 5 mg/mL, but become expanded at Brij 58 concentrations of 2.5 mg/mL and below. RNA-free nucleoids,

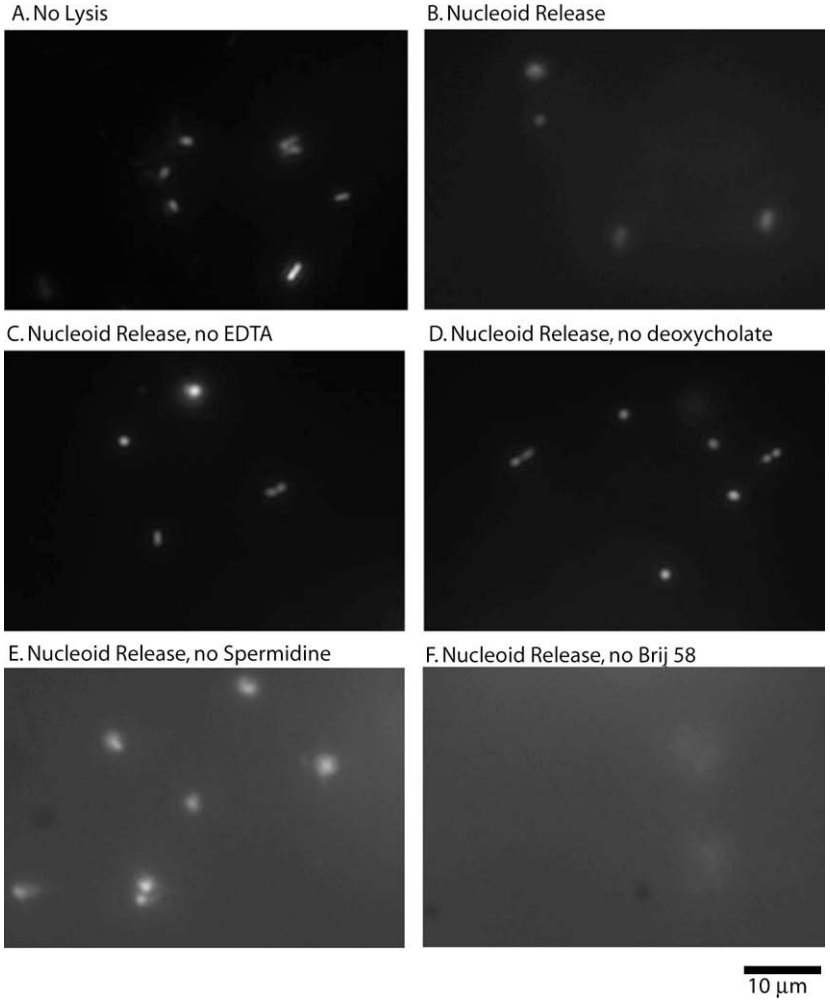
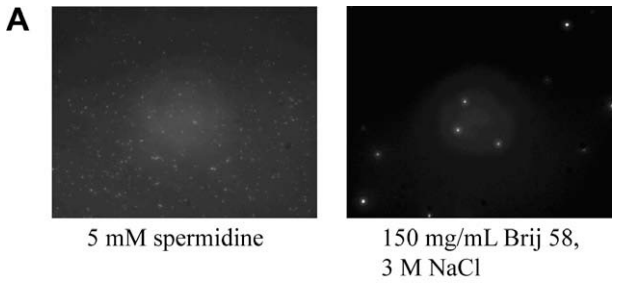


Fig. 1. Spermidine and Brij 58 contribute to *E. coli* nucleoid compaction after cell lysis. Fluorescent micrographs showing: (A) Unlysed *E. coli* cells. (B) Nucleoids released from exponential phase *E. coli* cells in complete stabilization solution. (C–F) Nucleoids released from exponential phase *E. coli* cells in stabilization solution lacking EDTA (C), sodium deoxycholate (D), spermidine (E), or Brij 58 (F).



B

NaCl (M)	Brij 58 (mg/mL) 100	150	200
1	D	D	semi
2	D	semi	C
3	D	C	C
4	D	C	C
5	C	C	C

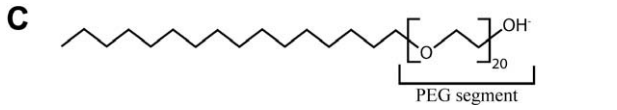


Fig. 2. Spermidine and Brij 58 can condense naked *E. coli* chromosomal DNA. (A–B) Brij 58-mediated compaction of DNA at several sodium chloride concentrations. D, dispersed DNA; C, compacted DNA; semi, partially decompacted DNA. (C) Molecular structure of Brij 58.

produced both *in vivo* and after cell lysis were not compacted at Brij 58 concentrations below 50 mg/mL. However, a Brij 58 concentration of 50 mg/mL induced compaction of RNA-free nucleoids to sizes that were comparable to native nucleoids. These results demonstrate Brij 58 participates in nucleoid compaction and stabilization after cell lysis, and that the removal of RNA from the nucleoid changes the compacting forces exhibited by Brij 58.

3.3. Protein-free nucleoids and control nucleoids behave similarly with respect to Brij 58-mediated compaction

Brij 58-mediated compaction of protein-free nucleoids (prepared either *in vivo* by treatment with chloramphenicol or following cell lysis by proteinase K digestion) was examined to investigate the role of NAPs in nucleoid compaction. We hypothesized that the removal of NAP-mediated compaction forces would require higher levels of Brij 58 for nucleoid compaction. We found native nucleoids, chloramphenicol-treated nucleoids and proteinase K-treated nucleoids all exhibited similar compaction characteristics in the presence of Brij 58 (Table 1), which indicated that NAPs do not play a key role in nucleoid compaction.

3.4. Nucleoids isolated under low- and high-salt conditions are similar in size

To further investigate whether NAPs compact chromosomal DNA, we prepared isolated nucleoids using both high- and low-salt procedures and observed the size of the compacted region using transmission electron microscopy. Nucleoids prepared using the high-salt isolation procedure lack nearly all nucleoid-associated proteins except RNA polymerase [30], while nucleoids prepared with the low-salt procedure retain these proteins [10]. We observed no significant difference in the area of the compacted region of nucleoids with and without NAPs (Fig. 3). These results corroborate our previous findings that indicate removal of NAPs from the chromosome does not affect the level of compaction.

4. Discussion

The effects of macromolecular crowding on naked DNA molecules in solution have been characterized previously [27,29,31]. Large DNA molecules are known to collapse in the presence of macromolecular concentrations similar to the concentration of Brij 58 required in these studies to compact fragmented chromosomal DNA, provided the polymer solution also contained an adequate ionic strength to shield the charges between DNA chains. Here, we show that Brij 58 is participating in nucleoid preparations not only as a detergent but also as a molecular crowding agent. Because Brij 58 is extensively used in nucleoid preparations we predict that macromolecular crowding plays a more extensive role in nucleoid condensation than previously realized.

We propose that chromosomal DNA can be represented as a large polyelectrolyte in solution with macromolecules. Nascent transcripts can be viewed essentially as branches emanating from the DNA polyelectrolyte backbone. These RNA branches increase both the branch density and molecular weight of the polymer. As a macromolecular crowding agent, the Brij 58 molecules decrease the solvent quality; therefore the repulsive forces between DNA segments compete with and are outweighed by the repulsive forces between the DNA and the solvent. Under these conditions, the DNA with associated RNA branches assumes a compressed random coil conformation.

This model can account for the RNase-sensitive constraint observed in both high- and low-salt nucleoid preparations, since it is known that nascent transcripts remain associated with the DNA under both conditions [10,15]. In addition, our model explains studies by both Cunha et al. and Murphy and Zimmerman, which found that nucleoids assumed a compact configuration at much lower concentrations of PEG than previously determined for naked DNA molecules (PEG concentrations of ~25 mg/mL versus ~120 mg/mL) [9,10,29]. Our model can also account for seemingly contradictory observations regarding the behavior of chloramphenicol-treated and rifampicin-treated nucleoids. Both treatments reduce transitional linkages between the nucleoid and the cell membrane resulting from simultaneous translation and membrane insertion as translation is inhibited by both chemicals. However in chloramphenicol-treated cells the result is strong nucleoid compaction in nucleoid preparations, whereas in rifampicin-treated cells nucleoid decompaction occurs upon lysis. According to our model, a high level of compaction would occur for chloramphenicol-treated nucleoids as the RNA branches remain associated with the DNA while the number of linkages is reduced. On the other hand, RNA would not remain associated with rifampicin-treated nucleoids, decreasing the molecular weight and branch density of the nucleoid, and result-

Table 1
Average cross-sectional area of treated nucleoids.

Brij 58 (mg/mL)	Control	Rifampicin	RNase A-treated	Chloramphenicol	Proteinase K-treated
1	8.05 ± 5.29 (n = 14)	8.85 ± 4.38 (n = 13)	11.76 ± 6.02 (n = 18)	7.56 ± 4.16 (n = 14)	10.31 ± 4.03 (n = 24)
2.5	6.45 ± 2.35 (n = 15)	9.43 ± 3.82 (n = 16)	7.27 ± 3.32 (n = 22)	7.77 ± 2.96 (n = 12)	6.87 ± 4.03 (n = 24)
5	2.01 ± 0.68 (n = 18)	6.16 ± 3.24 (n = 23)	5.45 ± 2.96 (n = 30)	4.03 ± 1.77 (n = 16)	4.50 ± 2.58 (n = 17)
10	2.11 ± 0.95 (n = 23)	5.17 ± 4.95 (n = 29)	6.33 ± 2.93 (n = 10)	3.33 ± 1.33 (n = 15)	1.89 ± 0.77 (n = 20)
25	2.16 ± 1.09 (n = 12)	6.61 ± 4.02 (n = 19)	4.10 ± 2.52 (n = 16)	2.35 ± 0.90 (n = 21)	2.25 ± 0.65 (n = 12)
50	2.70 ± 2.33 (n = 18)	2.62 ± 1.28 (n = 26)	2.13 ± 1.84 (n = 16)	2.35 ± 2.27 (n = 11)	3.10 ± 2.34 (n = 17)

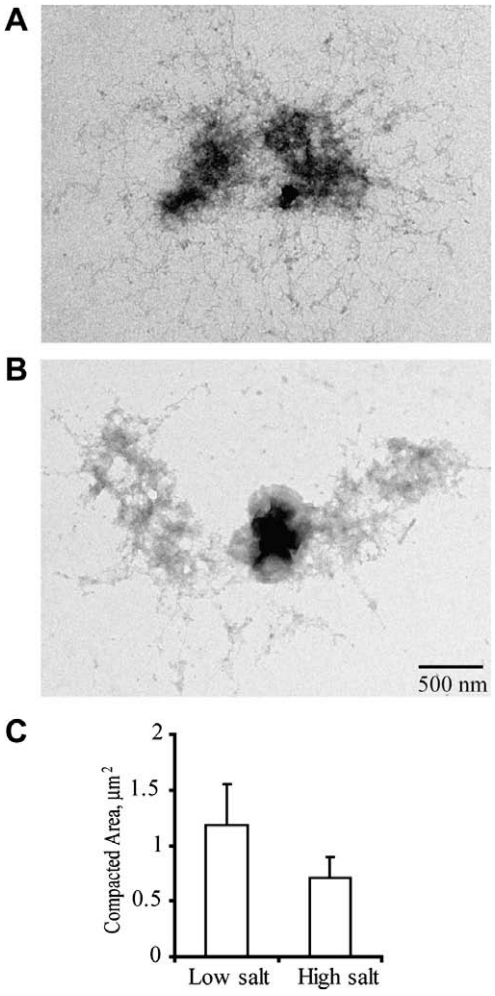


Fig. 3. Condensed *E. coli* nucleoids from high- and low-salt preparations are similar in size. (A) Electron micrograph of representative high-salt nucleoid; (B) electron micrograph of representative low-salt nucleoid. (C) Average area of condensed region of isolated nucleoids in electron micrographs. Error bars represent the standard deviation for $n = 8$ and $n = 6$ replicates of low- and high-salt nucleoids, respectively.

ing in a more dispersed configuration of the DNA in dilute polymer solutions. Our results are consistent with recent studies by Cabrera et al. [32]. The authors showed that when the RNA content of the *E. coli* nucleoid was decreased *in vivo* by either rifampin treatment or inhibition of rRNA synthesis, the nucleoid expanded. Importantly, the results presented here show it is likely the association of nascent transcripts rather than the process of transcription that causes nucleoid compaction, as active transcription is not occurring from the released nucleoids in this study.

We argue against a role for NAPs in nucleoid compaction as we hypothesize that the molecular weight and branch density of actively transcribing chromosomes would provide a strong compaction force. Consistently, we show that chloramphenicol-treated and proteinase K-treated nucleoids behave similarly to native nucleoids in regard to macromolecular crowding-mediated compaction. In addition, our results show no statistically significant difference in the sizes of nucleoids prepared using high- versus low-salt isolation procedures.

5. Conclusions

Nucleoid condensation in bacterial cells is dominated by the physical characteristics of the structure of the DNA, rather than biochemical factors such as nucleoid-associated proteins or specific RNA molecules. The results presented here showed that Brij 58 serves a previously unrecognized role as a macromolecular crowding agent in nucleoid preparations. We found that alterations to the molecular weight and branch density of the chromosome by RNA removal affect Brij 58-mediated DNA condensation, and these changes could explain the previously observed RNase-sensitive constraint. In addition, we showed that the removal of NAPs from bacterial nucleoid preparations did not significantly affect nucleoid size. We conclude that while NAPs most likely play a role in the structure and organization of the nucleoid, they do not serve as major compaction agents for chromosomal DNA in cells.

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References

[1] L.D. Murphy, S.B. Zimmerman, Multiple restraints to the unfolding of spermidine nucleoids from Escherichia coli, J. Struct. Biol. 132 (2000) 46–62.
[2] O. Stonington, D. Pettijohn, Folded Genome of *Escherichia coli* Isolated in a Protein-DNA-Rna Complex, Proc. Natl. Acad. Sci. USA 68 (1971) 6–9.
[3] D.J. Jin, J.E. Cabrera, Coupling the distribution of RNA polymerase to global gene regulation and the dynamic structure of the bacterial nucleoid in *Escherichia coli*, J. Struct. Biol. 156 (2006) 284–291.
[4] J. Pelta, F. Livolant, J.L. Sikorav, DNA aggregation induced by polyamines and cobalthexamine, J. Biol. Chem. 271 (1996) 5656–5662.
[5] I. Baeza, P. Gariglio, L.M. Rangel, P. Chavez, L. Cervantes, C. Arguello, C. Wong, C. Montanez, Electron Microscopy and Biochemical Properties of Polyamine-Compacted DNA, Biochemistry 26 (1987) 6387–6392.
[6] H. Oana, K. Tsumoto, Y. Yoshikawa, K. Yoshikawa, Folding transition of large DNA completely inhibits the action of a restriction endonuclease as revealed by single-chain observation, FEBS Lett. 530 (2002) 143–146.
[7] K. Tsumoto, F. Luckel, K. Yoshikawa, Giant DNA molecules exhibit on/off switching of transcriptional activity through conformational transition, Biophys. Chem. 106 (2003) 23–29.
[8] K. Yoshikawa, M. Takahashi, V.V. Vasilevskaya, A.R. Khokhlov, Large discrete transition in a single DNA molecule appears continuous in the ensemble, Phys. Rev. Lett. 76 (1996) 3029–3031.
[9] S. Cunha, C.L. Woldringh, T. Odijk, Polymer-mediated compaction and internal dynamics of isolated *Escherichia coli* nucleoids, J. Struct. Biol. 136 (2001) 53–66.
[10] L.D. Murphy, S.B. Zimmerman, Isolation and characterization of spermidine nucleoids from *Escherichia coli*, J. Struct. Biol. 119 (1997) 321–335.
[11] C.L. Woldringh, P.R. Jensen, H.V. Westerhoff, Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces?, FEMS Microbiol. Lett. 131 (1995) 235–242.
[12] S.B. Zimmerman, Cooperative transitions of isolated *Escherichia coli* nucleoids: Implications for the nucleoid as a cellular phase, J. Struct. Biol. 153 (2006) 160–175.
[13] S.B. Zimmerman, L.D. Murphy, Release of compact nucleoids with characteristic shapes from *Escherichia coli*, J. Bacteriol. 183 (2001) 5041–5049.
[14] P. Dworsky, Unfolding of chromosome of *Escherichia coli* after treatment with rifampicin, Z. Allg. Mikrobiol. 15 (1975) 243–247.
[15] P. Dworsky, M. Schaechter, Effect of rifampin on the structure and membrane attachment of the nucleoid of *Escherichia coli*, J. Bacteriol. 116 (1973) 1364–1374.
[16] L.D. Murphy, S.B. Zimmerman, Hypothesis: the RNase-sensitive restraint to unfolding of spermidine nucleoids from *Escherichia coli* is composed of cotranslational insertion linkages, Biophys. Chem. 101 (2002) 321–331.
[17] L.D. Murphy, S.B. Zimmerman, Macromolecular crowding effects on the interaction of DNA with *Escherichia coli* DNA-binding proteins - a model for bacterial nucleoid stabilization, Biochim. Biophys. Acta 1219 (1994) 277–284.
[18] R. Brunetti, G. Prosseda, E. Beghetto, B. Colonna, G. Micheli, The looped domain organization of the nucleoid in histone-like protein defective *Escherichia coli* strains, Biochimie 83 (2001) 873–882.
[19] D.G. Gibson, G.A. Benders, C. Andrews-Pfannkoch, E.A. Denisova, H. Baden-Tillson, J. Zaveri, T.B. Stockwell, A. Brownley, D.W. Thomas, M.A. Algire, C. Merryman, L. Young, V.N. Noskov, J.I. Glass, J.C. Venter, C.A. Hutchison, H.O.

Smith, Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome, Science 319 (2008) 1215–1220.
[20] J.I. Glass, N. Assad-Garcia, N. Alperovich, S. Yooseph, M.R. Lewis, M. Maruf III, C.A. Hutchison III, H.O. Smith, J.C. Venter, Essential genes of a minimal bacterium, Proc. Natl. Acad. Sci. USA 103 (2006) 425–430.
[21] C. Lartigue, S. Vashee, M.A. Algire, R.Y. Chuang, G.A. Benders, L. Ma, V.N. Noskov, E.A. Denisova, D.G. Gibson, N. Assad-Garcia, N. Alperovich, D.W. Thomas, C. Merryman, C.A. Hutchison, H.O. Smith, J.C. Venter, J.I. Glass, Creating bacterial strains from genomes that have been cloned and engineered in yeast, Science 325 (2009) 1693–1696.
[22] A. Moya, R. Gil, A. Latorre, J. Pereto, M.P. Garcillan-Barcia, F. de la Cruz, Toward minimal bacterial cells: evolution vs. design, FEMS Microbiol. Rev. 33 (2009) 225–235.
[23] N. Rusk, Synthetic Life, Nat. Methods 6 (2009) 33.
[24] R.B. Roberts, P.H. Abelson, D.B. Cowie, E.T. Boulton, R.J. Britten, Studies of Biosynthesis in *Escherichia coli*, Carnegie Institute of Washington, Washington, DC, 1955.
[25] T. Kornberg, A. Lockwood, A. Worcel, Replication of the *Escherichia coli* chromosome with a soluble enzyme system, Proc. Natl. Acad. Sci. USA 71 (1974) 3189–3193.
[26] L. Postow, C.D. Hardy, J. Arsuaga, N.R. Cozzarelli, Topological domain structure of the *Escherichia coli* chromosome, Genes Dev. 18 (2004) 1766–1779.
[27] R. de Vries, Flexible polymer-induced condensation and bundle formation of DNA and F-actin filaments, Biophys. J. 80 (2001) 1186–1194.
[28] H.L. Frisch, S. Fesciyan, DNA phase transitions - Psi transition of single coils, J. Polym. Sci. Part C: Polym. Lett. 17 (1979) 309–315.
[29] V.V. Vasilevskaya, A.R. Khokhlov, Y. Matsuzawa, K. Yoshikawa, Collapse of single DNA molecule in poly(ethylene glycol) solutions, J. Chem. Phys. 102 (1995) 6595–6602.
[30] D.E. Pettijohn, K. Clarkson, C.R. Kossman, O.G. Stonington, Synthesis of ribosomal RNA on a protein-DNA complex isolated from bacteria: a comparison of ribosomal RNA synthesis *in vitro* and *in vivo*, J. Mol. Biol. 52 (1970) 281–300.
[31] D. Pastré, L. Hamon, A. Mechulam, I. Sorel, S. Baconnais, P.A. Curmi, E. Le Cam, O. Pietrement, Atomic force microscopy imaging of DNA under macromolecular crowding conditions, Biomacromolecules 8 (2007) 3712–3717.
[32] J.E. Cabrera, C. Cagliero, S. Quan, C.L. Squires, D.J. Jin, Active transcription of rRNA operons condenses the nucleoid in *Escherichia coli*: Examining the effect of transcription on nucleoid structure in the absence of transertion, J. Bacteriol. 191 (2009) 4180–4185.