Strong intranucleoid interactions organize the *Escherichia coli* chromosome into a nucleoid filament

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The stochasticity of chromosome organization was investigated by fluorescently labeling genetic loci in live *Escherichia coli* cells. In spite of the common assumption that the chromosome is well modeled by an unstructured polymer, measurements of the locus distributions reveal that the *E. coli* chromosome is precisely organized into a nucleoid filament with a linear order. Loci in the body of the nucleoid show a precision of positioning within the cell of better than 10% of the cell length. The precision of interlocus distance of genomically-proximate loci was better than 4% of the cell length. The measured dependence of the precision of interlocus distance on genomic distance singles out intranucleoid interactions as the mechanism responsible for chromosome organization. From the magnitude of the variance, we infer the existence of an as-yet uncharacterized higher-order DNA organization in bacteria. We demonstrate that both the stochastic and average structure of the nucleoid is captured by a fluctuating elastic filament model.

Our quantitative measurements of *E. coli* nucleoid structure confirm this qualitative picture: The body of the nucleoid is linearly organized along the long-axis of the cell, implying that the nucleoid has a nearly-constant linear packing density, except for a short region, genomically *ter*-proximate, which connects the two arms of the chromosome (11). But in spite of the observation of a linear chromosome organization in both *E. coli* and *C. crescentus*, the mechanism which gives rise to this characteristic structure is unknown.

To probe the mechanism of nucleoid organization, we measure and analyze the position fluctuations of single loci and the correlation between the fluctuations of locus pairs within a unique conceptual framework. We consider locus positioning mechanisms which fall into two generic classes: *external* and *internal*. An *external mechanism* positions a locus directly, without acting through any other genetic loci in the nucleoid. For instance, a protein factor like FtsK, which is positioned at the septum and binds a *ter*-proximate locus, positions that locus by an *external mechanism*.

An *internal mechanism* positions a locus relative to another locus in the nucleoid. For instance, proteins like H-NS, which is a DNA bridging protein, and MukBEF, mediate DNA-DNA interactions and therefore structure the nucleoid by an internal mechanism (12). To differentiate between these two modes of positioning, we independently measure the single-locus position and the interlocus distance distributions. Loci that are strongly coupled by intranucleoid interactions are expected to exhibit correlated fluctuations from their mean positions, which is not the case if external positioning mechanisms are dominant*.

To quantitatively interpret these correlations and the locus positioning distributions more generally, we introduce a model of nucleoid structure: the Fluctuating Filament Model; see Fig. 4. This model includes intranucleoid interactions as well as two explicit mechanisms of external positioning: (i) cellular tethering of loci; and (ii) cellular confinement of the chromosome. The analysis of the experimentally determined locus distributions in the context of this model reveals that the nucleoid positioning in slow growing *E. coli* cells in G1 phase (before the initiation of segregation) is dominated by strong intranucleoid interactions. Strong external positioning occurs only at the poles. The precision of this positioning, its dependence on cell length, and the fine-scale features of the locus position distributions of *pole*-proximate loci, all strongly suggest that cellular confinement


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*Elaborate models can be proposed that cannot be simply characterized in this framework. For instance, positioning mechanisms can be proposed in which the position of the external chromosome tethers are correlated.*

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Fig. 1. Experimental schematic: The *E. coli* fiducial strains (IL01t, IL05, IL06) each carry two fiducial fluorescent loci (red and yellow foci) in addition to a probe locus (cyan lines). (A) A schematic map of the genomic location of the probes by strain. The three concentric rings represent the three fiducial strains. (B) The left and right arms of the *E. coli* chromosome are positioned on opposite sides of the nucleoid, with the origin at midcell. The terminus-proximate loci are positioned at both ends of the nucleoid, as well as in a crossing region that bridges the two poles of the nucleoid. External positioning mechanisms, which position a locus directly relative to the cell, are represented by *curly springs*. Internal positioning mechanisms, which position loci relative to one another, are represented by *zigzag springs*. (C) A typical composite image for the “IL06 12” strain.

plays the dominant role in external positioning in G1 phase. The observed high degree of internal ordering has implications for chromosome segregation and suggests that the *E. coli* chromosome is folded into a prokaryotic chromosome fiber.

**Results**

**Locus Distributions.** Single-locus position and interlocus distance distribution functions were generated from measurements of the locus positions. The distributions for the “IL01t C4” strain are shown in Fig. 2A, B. The distribution of loci positions in the body of the nucleoid (ter-distant) were found to be well-approximated by a Gaussian. To characterize the dependence of the distributions on genomic position, we fit the experimentally determined distributions to Gaussian distributions to determine the mean and variance as best-fit values. *oriC*-distant loci were characterized by asymmetric Gaussian-like distributions which decayed more steeply on the side closer to the cell pole. (See *lac* distribution in Fig. 2A.) For ter-proximate loci, cells showed two behaviors: Loci were either positioned at one of the poles of the nucleoid, or in an unpositioned state that was distributed uniformly between the poles of the nucleoid. (Please see *SI Text.*)

The structural significance of this crossing region is clear from the schematic drawing (Fig. 1B): The two poles of the nucleoid are connected by a low-packing-density fiber that stretches between them. We estimate the crossing region consists of just 8% of the genome and therefore has a packing density of just 1/10 that of the body of the nucleoid. This low packing density marks the crossing region as structurally distinct and therefore a potential target of remodeling. Although the genomic location of this region is always ter-proximate, it is stochastic.

**Mean Locus Position.** The mean position of loci is shown as a function of genomic position in Fig. 3A. As described previously, *oriC* is positioned at midcell and the left and right arms of the chromosome are positioned on opposing sides of the cell (11, 13). The most striking feature of this plot is that the mean position is linear in the genomic position, interpolating smoothly from ter-left at the left pole, through the origin at midcell, to ter-right at the right pole. This linear dependence implies that the nucleoid is linearly organized along the long-axis and has an approximately constant linear packaging density of 1.6 Mb/μm. This nucleoid structure is strikingly reminiscent of *C. crescentus*, where a similar linear organization was observed although with *ori* and *ter* positioned at opposing poles (8).

**Locus Variance.** The notion of locus positioning implies more than a mean value; it implies a precision. A locus uniformly distributed throughout the cell would have a mean position at midcell, but would not be considered “well-positioned.” The precision of positioning is quantified by the variance (σ) of the locus position distribution. In Fig. 3B, we plot the single-locus and the *oriC*-probe interlocus variance as a function of genomic position. The single-locus variance (blue) is roughly constant throughout the body of the nucleoid. All loci in the body are positioned with an accuracy of σ ~ 9% of the cell length. In Fig. 3B, we plot the interlocus variance with respect to *oriC* (green). In marked contrast to the single-locus variance, which is approximately constant throughout the body of the nucleoid, the interlocus variance is strongly dependent on the interlocus genomic distance between loci. The *oriC*-proximate loci are positioned with much greater precision (that is, smaller variance) with respect to *oriC* than with respect to the cell. The positions of *oriC* and *oriC*-proximate loci are therefore highly correlated. Although this result is not completely unexpected, our measurements reveal that the variance increases linearly with interlocus genomic distance:

\[
\sigma^2_{L} \propto \Delta L.
\]

This coupling is not unique to *oriC*, but most likely a general feature of nucleoid structure. All loci in the body of the nucleoid appear to be positioned with greater precision relative to their

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1. See *SI Text* The crossing region.
2. The interpretation of the variation in the crossing region is more subtle since these distributions are not well-approximated by a Gaussian distribution. Please see the discussion in *SI Text*, The crossing region.
3. The probe-lac interlocus variance shows the same trend. Please see *SI Text*, Intranucleoidal interactions are not unique to *oriC*. 

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neighbors than with respect to the cell, suggesting that intranucleoidal interactions play a central role in shaping nucleoid structure.

**Fluctuating Filament Model.** The qualitative conclusions about the role of intranucleoidal interactions in shaping nucleoid structure can be explored quantitatively in the context of an effective physical model. We introduce the Fluctuating Filament Model which represents the nucleoid as a fluctuating elastic filament, rather than resolving the detailed DNA conformation. (The Fluctuating Filament Model is shown schematically in Fig. 4A and is discussed in detail in SI Text, The Fluctuating Spring Model).

The filament is assumed to have a constant genome packing density $\eta$ (expressed in units of base pairs per micron) between ter-left and ter-right. The undeformed length of the nucleoid is $X_{\text{nucl}} = \text{genome length}/\eta$. The fluctuations of the nucleoid are described by an *effective elastic modulus*, $\gamma$, expressed in units of base pairs per micron squared. $\eta$ and $\gamma$ are assumed to completely characterize the intranucleoid interactions. In addition to internal organization, we confine the nucleoid to a region length $X_{\text{conf}}$ by forbidding DNA loci outside the region $X_-$ to $X_+$. We then compute the probability of different conformations assuming a Boltzmann Law: The probability of a given configuration is $p \propto \exp(-G)$, where $G$ is the total elastic energy of the Fluctuating Filament Model expressed in thermal units, $k_B T$. The model statistics are calculated by summing over nucleoid configurations, which is performed by Metropolis Monte Carlo Integration.

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**Model Results.** To illustrate the role of the positioning mechanisms in shaping chromosome structure, we have computed the distribution functions for an expository nucleoid structure that is organized by both tethering interactions and confinement, as well as intranucleoid interactions. These calculations are described in detail in SI Text, The Fluctuating Spring Model. We summarize the general features of the model in this section.

(i) The Fluctuating Filament Model generically recovers the linear dependence of the interlocus distance variance on genomic distance ($L$) for proximate loci $i$. The linear dependence of the interlocus variance is a generic signature of positioning by uniform nearest-neighbor interactions.$^9$

(ii) The single-locus variance is shaped by both external and internal interactions. At genomic positions that are tethered to the cell, the variance has a local minimum$^9$. The nucleoid elasticity determines the depth of these tethering-induced minima relative to the variance in the positioning of proximate loci.

(iii) Confined nucleoids ($X_{\text{nucl}} \geq X_{\text{conf}}$) can result in nearly constant single-locus variance$^{10}$ but, confinement manifests itself in the shape of the position distribution functions, which display a characteristic asymmetry: the distribution function decays more steeply on the side directly affected by confinement$^{10}$.

**Discussion**

**Intranucleoidal Interactions.** The observed linear dependence of the interlocus variance on interlocus genomic distance strongly suggests that intranucleoid interactions play a dominant role in shaping the *E. coli* nucleoid. This measurement alone argues strongly against multiple individually addressed (externally positioned) loci (1, 8), unless these tethered loci are all located at

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\*The crossing region is not included in the model.

\*\*See SI Text, Elastic constant need not be mechanical in nature.

\*\*\*See SI Text, The model results for the expository nucleoid structure.

\*\*\*\*See SI Text, The model results for the expository nucleoid structure.

\*\*\*\*\*See SI Text, Asymmetric distributions.
the poles of the nucleoid. Likewise, the single-locus variance appears to be at a minimum only at the cell poles, further supporting the argument that no loci in the body of the nucleoid are strongly positioned by tethering interactions. If loci in the body of the nucleoid are tethered, these interactions must either result in very weak positioning or be short-lived. These results do not imply that DNA tethering does not play a significant role in structuring the nucleoid at other steps in the cell cycle.***

The Case for Confinement. Two mechanisms of external positioning—polar tethering and confinement—could be acting at the poles of the nucleoid to reproduce the observed variance data. A number of arguments favor the confinement model: (i) The nucleoid is known to be confined by the cell and the size of the variance is commensurate with what is expected from confinement-induced positioning; (ii) If our analysis is repeated for cell populations grouped by length, the precision of cellular positioning decreases with cell length while the precision of relative intranucleoid positioning increases with cell length, as predicted in a confinement-based model.** Further- more, in very long cells, where replication initiation has been arrested, the nucleoid does not continue to grow with the cell and is not positioned at the poles of the cell, as would be predicted by polar tethering. Analogously, reducing the scale of the nucleoid by condensation also leads to the loss of nucleoid positioning (14); (iii) The distribution of pole-proximate loci exhibit the characteristic asymmetry predicted by confinement-induced positioning. (See the observed distributions in Fig. 4B and in SI Text, Quantitative analysis of locus histograms.) In short, there is no compelling argument to propose the existence of a yet-to-be-identified factor to position the terminus when cellular confinement is sufficient to position the nucleoid.

The G1 structure of the nucleoid is predicted by a simple model dominated by: (i) intranucleoid interactions that position the vast majority of loci; and (ii) cellular confinement which positions the poles of the nucleoid. The model is specified by just two parameters characterizing the nucleoid: the positioning modulus \( \gamma = 57 \pm 1 \) genomes/cell length\(^2\) characterizes the intranucleoid interactions, and the packing density \( \eta = 1.16 \pm 0.01 \) genomes/cell length\(^2\). Two additional parameters, limiting coordination of the nucleoid-to-cytoplasmic cytoplasm, describe the cell. (A cytoplasmic region length \( X \text{conf} = 0.88 \pm 0.01 \) cell lengths is accessible to the nucleoid.) The model parameters were determined by a chi-squared fit to the means and variances of the locus distributions\(^11\). The model not only recapitulates the observed dependence of the mean and variance on locus position as a function of genomic position (Fig. 3), but predicts the entire single-locus and interlocus distribution functions for all loci in the body of the nucleoid. Fig. 4B compares the observed and predicted distribution functions for three loci. A particularly significant feature of this plot is the prediction of a fine-scale feature of the distribution: the confinement-induced asymmetry characterized by a steep decay on the confinement-proximate side of the distribution (left side of E10 and right side of A11). This feature is not fit in the determination of the optimal model parameters but is rather a prediction of the model.

Higher-Order DNA Structure. Although we have argued that nucleoid positioning— for instance, the positioning of oriC at midcell—is predicted by the Fluctuating Filament Model, the two key structural features of this model (constant linear packing density and the intranucleoidal interactions) have not been derived from a microscopic model that explicitly treats the polymeric nature of the chromosome. To interpret the significance of our results, we now explicitly consider the predictions of an equilibrium polymer model. In a concentrated polymer solution, the Flory theorem predicts that the effects of excluded volume on the equilibrium polymer conformation can be ignored (15). In a polymer model, the effective elasticity is

\[
\gamma = \frac{3 \eta_{\text{fiber}}}{2 \ell_p} \tag{2}
\]

where \( \eta_{\text{fiber}} \) is the basepair packing density of the chromosome fiber and \( \ell_p \) is the bend persistence length\(^12\). Higher-order structure (or lengthwise condensation (18)) condenses the DNA locally via genomically-proximate interactions (as opposed to long-ranged interactions which bridge genomically-distant sequences) to form a chromosome fiber. These models are still polymer models, but the chromosome fiber has a larger basepair packing density per unit fiber length \( \eta_{\text{fiber}} \) than double-stranded DNA (dsDNA). For instance, in eukaryotes 10 and 30 nm fiber are both examples of higher-order DNA structure. In the absence of higher-order structure, we expect the effective elasticity to be approximately the observed envelope structure of dsDNA: \( \gamma = 90 \text{ kb/}\mu^2 \), which is between two and three orders of magnitude smaller than the observed effective elasticity \( \gamma = 3 \times 10^4 \text{ kb/}\mu^2 \). To match the observed \( \gamma \), the persistence length of DNA would have to be reduced by a factor of 300 to half a base pair! Neither the addition of a large number of nucleoid associated proteins (NAPs) which bridge and bend the DNA (12) nor supercoiling can explain such a large discrepancy in \( \gamma \), if the polymer structure is equilibrated. The large effective elasticity is therefore evidence for higher-order structure.

One possible structural realization of a prokaryotic chromosome fiber is an ordered stack of plectonomic loops, drawn schematically in the inset of Fig. 4A. It is assumed that the genomic positions of the plectonemes are essentially frozen in and cannot equilibrate. If these plectonomic loops corresponded to the 10 kb topological domains reported to structure the \( E. \coli \) chromosome (19) and the plectoneme loop stems are spaced by 100 base pairs, the resulting chromosome fiber would have 100-fold larger fiber packing density \( \eta_{\text{fiber}} \) than dsDNA and therefore a 100-fold increase in the effective elasticity \( \gamma \), approaching the observed value. Intriguingly, a back-of-the-envelope estimate of the width of such a fiber suggests that it is on order 0.5 \( \mu \)m, roughly the same value as the cell and therefore the nucleoid filament itself\(^10\). This short, thick fiber is poorly approximated by Eq. 2: the nucleoid is better modeled by an elastic filament model than a polymer model.

The success of the Fluctuating Filament Model in describing the structure of the \( E. \coli \) nucleoid suggests that the nucleoid may be more analogous to the precisely structured eukaryotic mitotic chromosome than to a eukaryotic interphase chromosome. The ordered structure of the nucleoid between replication cycles may have important implications for understanding chromosome segregation. Since the polymer model fails to describe the G1 structure of prokaryotic chromosomes, it is important to reconsider our assumptions about the nature of chromosome segregation in light of our new structural insights into chromosome structure.

Conclusion. In this paper we characterize the stochasticity of the \( E. \coli \) nucleoid structure between replication cycles. We measured both single-locus position and interlocus distance distribution.

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*In fact, ter is known to be tethered at the z-ring in late S-C phase.

**See SI Text, The dependence of the model parameters on cell length.

***See SI Text, The Fluctuating Spring Model fit to experimental data...

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10Recent studies have suggested that the Flory Theorem may fail to describe tightly confined polymers and that confinement may play a role in segregating and organizing the chromosome (7, 16). On the other hand, rigorous tests of the Flory Theorem have demonstrated that the theorem holds in a regime we believe to be relevant for describing unstructured DNA in the cell (17). As we consider larger fiber packing densities, these filaments are no longer described by Eq. 2 since the monomer size becomes significant in relation to the confinement radius of the nucleoid.

11Please see the estimate in SI Text, Polymer interpretation of the nucleoid stiffness.
Analysis of single-locus position fluctuations revealed that all loci in the body of the nucleoid are positioned with a precision of roughly 10% of cell length with respect to the cell. The analysis of the coupling of genomically proximate loci reveals that loci are precisely positioned relative to their neighbors (<4% cell length), suggesting that the nucleoid organization is dominated by intranucleoid interactions. In particular, we demonstrate that the variance of the interlocus distance distribution grows linearly with their genomic separation, which is the signature dependence of a conformation structured by nearest-neighbor interactions. To investigate the role of these internal interactions, we introduce the Fluctuating Filament Model which approximates the E. coli nucleoid as a confined, elastic filament with a constant DNA packing density. The nucleoid is characterized by just two parameters: the linear packing density which governs the mean position of loci, and an effective elasticity which characterizes the fluctuations. The quantitative agreement of nucleoid structure with the Fluctuating Filament Model suggests that the most natural structural analogue of the nucleoid maybe the mitotic rather than the interphase eukaryotic chromosome. The precise internal order, relative to that predicted by polymer models, is strong evidence for higher-order DNA structure. We anticipate that these unique insights into the structure of the nucleoid between replication cycles will have important implications for our understanding of prokaryotic chromosome segregation.

Methods

The methods are described in detail in SI Text, Experimental Methods. To probe the nucleoid structure in live cells, we adapted both the Fluorescent Repressor Operator System (FROS) (20) and the ParB-parS system (21) to visualize three genetic loci concurrently. Both labeling technologies allow genomic loci to be visualized by the aggregation of fluorescently labeled proteins at an ectopic sequence introduced at the locus of interest. Our fiducial strains carry two fiducial labels, one at oriC and the other at an oriC-distant locus. The number of oriC loci is used to determine whether the cells have initiated the segregation process (20, 21). The second fiducial label is used to determine the left-right polarity of the cell (11, 13). (See Fig. 1 for schematic maps of the fiducial strains.) From the fiducial strains, we generated a collection of probe strains by inserting the parS sequence at a random, probe locus using a mariner-based transposon (8, 22). The snapshot technique was used to generate a population-based distribution of locus position. The microscopy is described in more detail in SI Text, Microscopy and growth conditions. Cells are segmented from a phase contrast image, and loci in the three fluorescence channels (RFP, YFP, and CFP) are identified, counted, and their position determined along the long and short axes of the cell.

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Supporting Information

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SI Text

Experimental Methods. Fiducial strains. The fiducial strains IL06, IL05, and IL01t each carry ectopic lacO and tetO operator arrays.

- IL06 carries an ori-proximate lacO array (15 kb counter-clockwise from oriC) and a ter-proximate tetO array (50 kb clockwise from dif). (IL06 was the gift of the Sherratt Lab (S1).)
- IL05 carries an ori-proximate tetO array (15 kb counter-clockwise from oriC) and a ter-proximate lacO array (210 kb clockwise from dif). (IL05 was the gift of the Sherratt Lab (S1).)
- IL01t carries an ori-proximate lacO array (15 kb counter-clockwise from oriC) and a lac-proximate tetO array. To build IL01t, we used P1 transduction to transfer the tetO array at the lac operator from AB215 (Gift of Aude Bourinau, Kleckner Lab) into IL01 (Sherratt Lab). (We used the Sauer Lab protocol from P1 transduction (S2.).)

Probe strains. To introduce a probe locus, we used a mariner-based transposon to randomly insert a parS sequence cassette into the genome. (Please see pJSM2048: transposon-based parS insertion system for details on the transposon.) About 100 strains were generated, of which only 15 have been characterized in detail. Please see Table 1 for the locations of the transposon. The probe locus was originally introduced into IL06. Once the transposons were mapped, the probe locus from the strain of interest was transferred from this IL06 to the other fiducial strains by P1 transduction (S2.).

Transposon mapping. To map the genomic position of the transposon insertions, we used inverse PCR (S3) to amplify the insert-proximate genome sequence. This sequence was then sequenced using an internal primer.

Inverse PCR protocol.

- Genomic DNA was prepared from E. coli using (and as described by) the Wizard® Genomic DNA Purification Kit (Promega Corporation).
- 5 µg of DNA was digested with 1 µL of NlaIII restriction enzyme (New England Biolabs) for 6 h at 37°C.
- The DNA was then cleaned using a QIAquick PCR Purification Kit (QIAGEN Inc.) as described in the manual. The DNA was eluted in 50 µL of QIAGEN buffer EB (10 mM Tris-Cl, pH 8.5).
- The DNA was then self-ligated in a 100 µL volume using 1 µL of T4 DNA ligase (New England Biolabs) over night at 4°C.
- The DNA was then cleaned using a QIAquick PCR Purification Kit (QIAGEN) as described in the manual. The DNA was eluted in 50 µL of buffer EB.
- 6 µL of DNA was then amplified in a 50 µL PCR reaction using taq DNA polymerase (New England Biolabs). The concentrations of buffers and reactants are as recommended by NEB. The PCR primers were ip4 (GGGGGGGGGGGAAAGCCACG) and ip8 (GTCATAGCCGGCCCGAAGTTC). PCR reaction conditions were:

  1. 3 min at 94°C.
  2. 45 sec at 94°C.
  3. 30 sec at 55°C.
  4. 3 min at 72°C.
  5. go to step 2 39 times.
  6. 10 min at 72°C.

- The presence of DNA bands were checked by gel electrophoresis.
- PCR products with bands were then cleaned (as described above) and sent for sequencing using internal primer ip3 (AATCTCTGATGTTAATGC).
- To map the probe locations, the DNA sequences were aligned to the MG1655 E. coli genome sequence.

Plasmids.

pAW34: Expression system plasmid.

Three fluorescent loci were simultaneously labeled using a combination of the Fluorescent Repressor Operator System (FROS) (S4) and the ParB-parS system (S5). The plasmid pAW34 was constructed to drive the three fluorescent fusions, mCherry-lacI, tetR-EYFP, and CFP-parB, with the L-arabinose inducible promoter PBAD.

To construct pAW34, we started with fusion expression plasmid pLAU53 (the gift of the Sherratt Lab (S4)). pLAU53 is a pBR322 (∼16 copies) plasmid with L-arabinose inducible expression of polycistronic lacI-ECFP and tetR-EYFP. We replaced the lacI-ECFP gene with the mCherry-lacI, tetR-EYFP, and CFP-parB, with the L-arabinose inducible promoter PBAD.

pJSM2048: transposon-based parS insertion system.

We adapted the technique demonstrated by the Shapiro Lab of using mariner-based transposons to insert an ectopic labeling sequence into the genome at a random location (S6). We started with a transposon-based mutagenesis system, pSC189, developed by the Rubin Lab (S7). The parS sequence cassette originated from the pALA1073 (a gift of the Austin Lab). We cloned the chlorophenoloreceptor resistance gene (car from pNG54 N. Grindley) into the middle of the kanR gene in pALA1073 to create a CmR resistance parS cassette (and remove kanamycin resistance). This cassette was then subcloned into kanR in pSC189 (again to remove kanamycin resistance) to make pJSM2048. To demonstrate the function of this construct, the pJSM2048 was conjugated from BW19851 (which expressed the pir gene) to IL06 (pir−) in which the plasmid cannot replicate. (Please see ref. S7 for the conjugation protocol.) The resulting strain was shown to be chlorophenolocult but not ampicillin resistant, demonstrating only the transposon had been integrated into the genome (and not the ampicillin resistant backbone of pJSM2048). An annotated schematic of pJSM2048 is shown schematically in Fig. S1.

Microscopy and growth conditions. Cells for microscopy were grown to OD600 of 0.3 at 30°C in M9 medium with 0.2% glycerol, 1 mM MgSO4, supplemented with amino acids (100 µg/mL Arginine, Histidine, Leucine, Theonoine, Proline) and 10 µg/mL thiamine. The doubling time for the cells was roughly 110 min. The distribution of cell length for cells containing only a single copy of the labeled loci is shown in Fig. S2.
For induction, cells were grown with 0.02% L-arabinose and supplemented with 0.5 mM IPTG or 40 ng/mL anhydrotretracycline (to reduce repressor binding, without compromising focus formation (S1)) for 30 min. (The doubling time for the induced cells was roughly 115 min.)

Cells were transferred to a 1.0% agarose layer containing phosphate-buffered saline for snap-shot microscopy (S1). The microscopy slides were prepared as follows:

- Microscope slides were wrapped with two layers of tape to form z-spacers on either side of a 1–2 cm gap for the agarose pad.
- The gap was then filled with melted agarose and a slide placed on top.
- The extra agarose was squeezed out by the application of pressure.
- The agarose was left to cool and set for about 5 min.
- The top slide was slid off the pad and the 10 µL of cells were spread on the pad using a pipette.
- The slides were allowed to dry of all visible media, occasionally respraying the cells as necessary.
- A coverslip was placed over the agarose pad.
- The coverslip was sealed with 1:1:1 vaseline:lanolin:paraffin.

The cells were imaged at room temperature on a Zeiss Axiovert 200M inverted microscope. The fluorescence source was an XCIte 120 mercury lamp. The images were captured using a Zeiss AxioCam MRm. The fluorescence exposure times were 1 sec.

Quantitative analysis of locus histograms. In our analysis, we analyze the precision of positioning by computing the mean and variance of the positioning distributions. Instead of the direct computation of the mean and variance from the raw coordinates of the loci, we advocate fitting the distributions to Gaussian distributions. This technique allows the characteristics of typical members of the population to be quantified while minimizing the effect of outliers. These outlying data points have a number of sources: (i) A fraction of cells are either missegmented or the locus number is under counted. (ii) A small number of cells show aberrant nucleoid structure. (For instance, in some cells, all three fluorescent loci are colocalized.) (iii) The detection of cell-cycle phase by counting loci implies that the cells we observe are in a mixed cell-cycle state because we cannot differentiate between cells that have initiated replication and those that have not, since the loci stay colocalized. (iv) These two phases of the cell initiated replication and those that have not, since the loci stay colocalized for roughly 10 min (S8). These positioned distributions. The structural significance of this unpositioned region is known to be anchored at the septum later in the cell cycle. Could the unpositioned loci correspond to the nonpositioned loci at the opposite pole and a “smoothed” box function between the positioned distributions at the ends of the loci:

\[
p(x,x_\pm,\sigma_\pm,\mathcal{P}_\pm) = \begin{cases} \mathcal{P}_\mathcal{L} \mathcal{G}(x;x_\pm,\sigma_\pm) & \text{LHS positioned loci} \\ \mathcal{P}_\mathcal{R} \mathcal{G}(x;x_\pm,\sigma_\pm) & \text{RHS positioned loci} \end{cases}
\]

where L/RHS are the left or right-hand-side of the cell respectively, \(\mathcal{P}_\mathcal{L}\) is the probability of being positioned on the right or left side respectively and

\[
\mathcal{P}_\text{cross} = 1 - \mathcal{P}_\mathcal{L} - \mathcal{P}_\mathcal{R}
\]

is the probability of being in the crossing region. For loci with a single peak on the ± side, \(\mathcal{P}_\pm\) is set to zero in the fit. The ter fit function is shown in Fig. S3.

Caveat for ter-proximate fits. It is important to note that the parameters obtained from the fit of the ter fit function are much more model dependent than the fits to the Gaussian distribution and therefore these parameters should be treated with more caution. In the main text, we have therefore shaded the regions of plots that use parameters derived from these fits to indicate where the crossing region complicates the interpretation of the distributions.

Supporting Results. The crossing region. Although ori-proximate loci are positioned in distributions that are well approximated by single Gaussian distributions, the distribution of ter-proximate loci are qualitatively different in nature, as illustrated in Fig. S4. (See Fig. S7A for the distributions of loci in the body of the nucleoid.) As exemplified by the ter-proximate fiducial locus in IL06 ("ter6"), ter-proximate loci can be positioned at the left or right pole of the nucleoid, or poorly positioned between poles. We call the distributions at the poles of the nucleoid “positioned” because they decay steeply on the pole-proximate side of the distribution. On the other hand, roughly 60% of the loci lie between these positioned distributions. The structural significance of this unpositioned region is clear: The schematic drawing of the nucleoid shows a low-packing-density “fiber” crossing the nucleoid connecting the left and right poles.

Since loci can be positioned at either pole or unpositioned in the crossing region, we know that the genomic position of the crossing state must be stochastic. We can estimate the probability of loci occupying this crossing region by fitting the distributions with Eq. S2. The crossing probability is shown in Fig. S3B as a function of genomic position. From the fit to the crossing probability, we can estimate the length of the crossing region by integrating over the crossing probability:

\[
\ell_\text{cross} = \int dL \mathcal{P}_\text{cross} = 8 \pm 4\% \text{Genome}
\]

implying a packing density of roughly 9 ± 4% of that in the body of the nucleoid.

There is an alternative explanation for unpositioned ter loci: The ter region is known to be anchored at the septum later in the cell cycle. Could the unpositioned loci correspond to the early migration of the ter region to midcell? Bates and coworkers have analyzed the oriC and ter loci positions in a time series in
synchronized *E. coli* cells (S8). Their data appear to suggest that *ter* does not move significantly before the observation of two oriC loci despite showing that newly divided cells still have unpositioned *ter* loci (supporting the crossing-region interpretation). On the other hand, we cannot rule out that a subset of the cells begin translocating *ter* to midcell early. Since the *ter* left (positioned at the left pole) and *ter* right (positioned at the right pole) are genomically connected, a crossing region must exist. But the genomic length of this region that we calculated in Eq. 5 must be considered a maximum because some of the unpositioned *ter* loci could correspond to the early movement of *ter*. Therefore the density contrast between the body of the nucleoid and the crossing region may be even greater than we have estimated.

**Intranucleoidal interactions are not unique to oriC.** In the main text, we claim that the intranucleoidal interactions (quantitatively characterized by fitting the oriC-probe interlocus distribution variance) are in fact generic and not unique to interactions with oriC. In the fiducial strain IL01t, we can analyze the interactions with the *lac* locus, which also exhibit a near linear increase in the variance with interlocus genomic distance, as shown in Fig. S5. (In principle, this analysis could be repeated for the second fiducial label in the IL05S and IL06 strains but the more complicated distribution of the *ter*-proximate fiducial labels make the quantitative analysis of interlocus distributions nontrivial, although the qualitative trend appears to be preserved.) The dependence of the variance on interlocus distance, rather than genomic position (excluding the crossing region) suggests that not only are intranucleoidal interactions generic, but that they are reasonably isotropic throughout the body of the nucleoid.

**The Fluctuating Spring Model.**

**Model motivation.**

In the main text, we introduced the Fluctuating Spring Model of the nucleoid using the language of statistical mechanics. We have referred to $\gamma$ as an elastic modulus, although we have not explicitly demonstrated that the observed fluctuations are thermal in nature, therefore excluding the rigorous identification of the fluctuations with mechanics. Instead, we treat statistical mechanics as a mnemonic for keeping track of the competing effects of external and internal positioning. Our stiffnesses are effective stiffness, measured in units of inverse length squared, rather than energy per length squared. The one-dimensional elastic modulus $k$ is typically defined in units of energy per unit length. Since we define the undeformed length in terms of genomic distance, we therefore incorporate the packing density into the constant that is directly measured:

$$\gamma = kN.$$  \[S5]\n
The spring constant between two loci that are separated by $N$ base pairs is

$$k = \gamma / N.$$  \[S6]\n
The effective elastic modulus or positioning modulus therefore has units of base pairs per unit length squared.

**Investigating the role of a backbone.**

The two-parameter model described in the discussion contains all the salient features of our model for nucleoid confinement. In this section we describe a more detailed model that adds one additional feature (and parameter) to the model: We introduce a plectoneme spring which couples the observed loci to a nucleoid backbone (responsible for intranucleoid interactions). In the next section, we demonstrate that the plectoneme springs are shown to be extremely stiff, implying that there is little distinction between the backbone (which feels the intranucleoid interactions) and the bulk of the nucleoid. The refined model is shown in Fig. S7A.

In our model, we ignore the crossing region of the nucleoid. See *Open questions* for further discussion of the significance of the crossing region.

**The Fluctuating Spring Model: Supplemental results.**

**The model parameters for the expository nucleoid structure.**

The expository model presented in the main text was designed to illustrate the competing effects of cellular tethering, confinement and intranucleoidal interactions in shaping the structure of the nucleoid. The model shown in the text was generated with the following parameters:

$$\gamma = 65.0 \text{ Genomes} \cdot \text{Cell Length}^{-2},$$  \[S7]\n
$$k_p = \infty \text{ Cell Length}^{-2},$$  \[S8]\n
$$k_{ori} = 400.0 \text{ Cell Length}^{-2},$$  \[S9]\n
$$X_{ori} = 0.5 \text{ Cell Length},$$  \[S10]\n
$$k_{ter} = 200.0 \text{ Cell Length}^{-2},$$  \[S11]\n
$$X_{ter} = 0.1 \text{ Cell Length},$$  \[S12]\n
$$X_{max} = 0.80 \text{ Cell Length},$$  \[S13]\n
$$X_+ = 1.0 \text{ Cell Length}.$$  \[S15]\n
where $\gamma$ is the nucleoid stretch modulus, $k_p$ is the plectonemic spring constant (which can be ignored in this example), $k_{ori}$ and $X_{ori}$ are the stiffness and cellular position of the external positioning spring at oriC, $k_{ter}$ and $X_{ter}$ are the stiffness and cellular position of the external positioning spring at *ter*-left, and $X_{max}$ is the undeformed nucleoid length. The nucleoid is confined to the region between $X_-$ and $X_+$ in the cell. We define the confinement length:

$$X_{conf} = X_+ - X_-.$$  \[S16]\n
The predicted variance for this model is shown in Fig. S6B.

**The model results for the expository nucleoid structure.**

To illustrate the role of the positioning mechanisms in shaping chromosome structure, we have computed the distribution functions for an expository nucleoid structure that is organized by tethering interactions at oriC and *ter*-left in addition to confinement, and intranucleoidal interactions. In the main text we make the case for which of these mechanisms shape the *E. coli* nucleoid, but for the moment we include a range of positioning mechanisms in order to discuss how each might affect the locus distribution. The expository nucleoid is shown schematically in Fig. S6A and the expected single-locus position and interlocus distances variances, as a function of genomic position, are shown in Fig. S6B.

The Fluctuating Spring Model generically recovers the linear dependence of the interlocus distance variance on genomic distance ($L$) for proximate loci. This result is generic since the interlocus interactions scale like $L^{-1}$ and are therefore always the dominant interaction for sufficiently small $L$. (See Fig. S6B.) In fact, the linear dependence of the interlocus distance variance is a generic signature of positioning by uniform nearest-neighbor...
interactions and follows from the central limit theorem, as discussed in *Elastic constant need not be mechanical in nature*. The slope of this linear dependence determines the effective elasticity of the nucleoid. Although internal interactions typically dominate the interlocus distance variance for proximate loci, external interactions affect the variance for distant loci, as discussed in Fig. S6B.

The single-locus position variance is shaped by both external and internal interactions. At genomic positions that are tethered to the cell, the variance has a local minimum (blue *`). Neighboring loci are less precisely positioned since successive intranucleoid links (represented by internal springs in the model) add to the variance. The nucleoid elasticity determines the depth of these tethering-induced minima relative to the variance in the positioning of proximate loci. The local maxima in the single-locus position variance (blue Δ) are maximally distant from the loci that are strongly positioned by external tethers.

In analogy to tethering, nucleoids that are strongly confined (∆X_m > ΔX_conf) are expected to have deep minima in the single-locus position variance at the genomic locations directly affected by confinement, like the ones close to the cell poles. On the other hand, weakly confined nucleoids (∆X_conf ≥ ∆X_m) can result in nearly constant single-locus variance, as discussed in Confinement leads to positioning that resembles polar tethering. But, even for weakly confined nucleoids, confinement manifests itself in the shape of the position distribution functions, which display a characteristic asymmetry: The distribution function decays more steeply on the side directly affected by confinement, as discussed in *Asymmetric distributions*.

The Fluctuating Spring Model predicts that the fluctuations of the nucleoid structure are key reporters of the mechanism of locus positioning. We therefore advocate for the analysis of the nucleoid structure are key reporters of the mechanism of locus positioning. We therefore advocate for the analysis of the nucleoid structure are key reporters of the mechanism of locus positioning.

**Confinement leads to positioning that resembles polar tethering.**

Confinement acts to constrain the positioning of the poles of the nucleoid by confining the nucleoid to a box of length ΔX_conf. With respect to the variance, the effect of confinement is analogous to tethers acting at the poles of the nucleoid. In Fig. S7B, we show the predicted single-locus variance for a range of confinement strengths. To compare these with the effect of polar tethering, we generated another set of nucleoid structures positioned by polar tethering only. The stiffness of *ter* springs are chosen to match the variance at oriC. As shown in Fig. S7, confinement tends to lead to slightly flatter distributions.

The confinement model parameters for the variances shown in Fig. S7B are:

\[ \nu = 67.0 \text{Genomes} \cdot \text{CellLength}^{-2}, \]  
**[S17]**

\[ k_p = \infty \text{CellLength}^{-2}, \]  
**[S18]**

\[ X_{\text{muc}} = \lbrace 0.7, 1.0, 2.0 \rbrace \text{CellLength}, \]  
**[S19]**

\[ X_- = 0.0 \text{CellLength}, \]  
**[S20]**

\[ X_+ = 1.0 \text{CellLength}, \]  
**[S21]**

where the three values for \( X_{\text{muc}} \) correspond to the weak, moderate, and strong confinement examples shown in Fig. S7B.

**The Fluctuating Spring Model fit to experimental data.**

To determine the optimal parameters for the Fluctuating Spring Model, we performed a \( \chi^2 \) fit of the observed single-locus and interlocus variances to those predicted by the Fluctuating Spring Model. Only data from the body of the nucleoid (probe strains from C10-A11) was fit since the crossing region is not represented in the model. The experimental data and fit are shown in Fig. S7C. The optimal model parameters were determined to be:

\[ \nu = 56.8 \pm 0.9 \text{Genomes} \cdot \text{CellLength}^{-2}, \]  
**[S22]**

\[ k_p = 2 \times 10^3 \pm 5 \times 10^2 \text{CellLength}^{-2}, \]  
**[S23]**

\[ X_{\text{muc}} = 0.86 \pm 0.01 \text{CellLength}, \]  
**[S24]**

\[ X_- = 0.04 \pm 0.01 \text{CellLength}, \]  
**[S25]**

\[ X_+ = 0.90 \pm 0.01 \text{CellLength}, \]  
**[S26]**

where \( \nu \) is the nucleoid stretch modulus, \( k_p \) is the plectonemic spring constant, and \( X_{\text{muc}} \) is the undeformed nucleoid length.

The value for the stretch modulus \( \nu \) is consistent with that obtained by direct analysis of the interlocus variance alone. The fact that the interlocus variance is consistently smaller than the single-locus variance for almost all observed locus spacings proves that intranucleoid interactions are the predominant mechanism structuring the nucleoid.

Intranucleoid interactions alone cannot position the nucleoid in the cell. The fit shown in Fig. S7 shows that moderate confinement (the undeformed length roughly equal the confinement length) is sufficient to give rise to the observed positioning. The fit also predicts that the plectonemes, introduced in the supplement only, are very stiff. This implies that the distinction between the backbone and the bulk of the nucleoid can be ignored in our analysis. (We therefore left out this distinction in the description of the model in the main text.) This data argues against a model where long loops, emanating from a stiff backbone, provide a significant contribution to the variance.

**Asymmetric distributions.**

As described in the previous section, the Fluctuating Spring Model is fit to the distribution variances alone, but the model predicts the entire distribution, not just the variance. In fact, the confinement-induced positioning model predicts fine-scale features of the observed distributions that are not fit in the model. The predicted distribution for three loci are shown in Fig. S4 in the main text. A tighter fit to these distributions can be achieved by fitting them directly as shown in Fig. S7D.

**The dependence of the model parameters on cell length.**

Does cell length matter? We have tacitly assumed throughout this paper that whatever mechanism acts to position loci in the cell acts with respect to the relative position in the cell, not the absolute position (S9). Clearly this assumption is at least in part justified: oriC is positioned at midcell regardless of whether midcell is located at 1.5 μm or 2 μm from the cell pole (S10). Is this a special feature of mean position of oriC, or is this apparent mechanism of relative positioning more generic?

To analyze the precision of positioning as a function of cell length, we divided the cells into three bins based on cell length Small (S: \( \ell_{\text{cell}} < 3 \mu m \)), Medium (M: \( 3 \mu m < \ell_{\text{cell}} < 3.4 \mu m \)), and Large (L: \( 3.4 \mu m < \ell_{\text{cell}} \)), histogrammed the locus positions, and analyzed the distributions. A positioning mechanism sensitive only to relative position would predict that the resulting distribution would be identical. The data, plotted in Fig. S8A, B show that the cell variance is cell length dependent, but shows...
opposite trends for the single-locus and interlocus variance. Fig. S8A shows that as cells get longer, the relative precision of positioning with respect to the cell is reduced: the variance for large cells is significantly greater than that for small cells (in units of cell length squared). On the other hand, the relative precision of the positioning of loci with respect to other loci in the nucleoid is increased: The variance of the interlocus distribution is smaller for larger cells (in units of cell length squared). These opposing trends are predicted by the nucleoid model. The model proposes that the nucleoid has an undeformed length \( X_{\text{nucl}} \) which is confined in a box length \( X_{\text{conf}} \). As the cell lengthens, it is natural to assume that the undeformed length of the nucleoid stays roughly constant, while the confinement length grows in proportion to cell length. Therefore we expect the scaling of the confinement strength to be

\[
\frac{X_{\text{nucl}}}{X_{\text{conf}}} \propto \ell^{-1}. \quad [S27]
\]

In other words, longer cells have weaker confinement. Similarly we would expect intranucleoidal interaction stiffness \( \gamma_{\text{abs}} \) to remain roughly the same in absolute units as the cell grows, but in units relative to cell length, the effective stiffness scales as

\[
\gamma \equiv \gamma_{\text{abs}} \ell^2 \propto \ell^{-2}. \quad [S28]
\]

In other words, the relative strength of intranucleoidal interactions are expected to increase with cell length even as the absolute strength remains constant.

By fitting the three length-binned populations to the nucleoid, we can measure how the strength of confinement and the effective stiffness of the nucleoid depend on cell length and compare this data to the trends predicted by confinement-induced nucleoid positioning 27 and 28. The plots in Fig. SSC, D confirm that experimental data show the predicted trend, although with a slighter weaker length dependence than predicted. The length dependence therefore provides a nontrivial test of the mechanism behind the confinement-induced nucleoid positioning model and explains the otherwise mysterious opposing trends in the dependence of single-locus and interlocus variance on cell length.

Note that a naive model of polar tethering would not predict the observed trend. The variance of both the single-locus and interlocus distributions would be expected to remain constant in absolute units since the stiffness of linear springs is independent of applied load.

**Supporting Discussion. Polymer interpretation of the nucleoid stiffness.** The polymeric structure of the chromosome suggests a polymeric interpretation of the intranucleoidal interactions. Long polymers behave like entropic springs with a spring constant of

\[
\gamma = \frac{3k}{2\ell_p}, \quad [S29]
\]

where \( k \) is the packing density in monomers per unit length and \( \ell_p \) is the persistence length. For comparison, we estimate the entropic elasticity for both naked double stranded DNA (dsDNA) and 10 nm fiber (S11):

\[
\gamma_{\text{measured}} \sim 3 \times 10^4 \text{kb} \cdot \mu\text{m}^{-2}, \quad [S30]
\]

\[
\gamma_{\text{dsDNA}} \sim 88 \text{kb} \cdot \mu\text{m}^{-2}, \quad [S31]
\]

\[
\gamma_{\text{10nm}} \sim 400 \text{kb} \cdot \mu\text{m}^{-2}, \quad [S32]
\]

where \( \gamma_{\text{measured}} \) is the elasticity measured in this paper. The elasticity of the nucleoid is between two and three orders of magnitude greater than that of dsDNA. The structural interpretation in the variance is nearly three orders of magnitude less than the variance predicted naively by dsDNA. Although this estimate ignores the role of confinement and excluded volume, it is a testament to the order of the nucleoid in comparison to the most simplistic polymer model.

Note that the observation that the nucleoid is divided into 10 kb topological domains (S12) suggests a natural mechanism for increased order: If 10 kb loops* are spaced by \( \sim 100 \) bp links, the packing density of dsDNA is increased by two orders of magnitude, increasing the predicted entropic stiffness to the observed value.

John Marko has recently considered the effect of “lengthwise condensation”—for instance the condensation of DNA into a 10 nm fiber—on the linking number (entanglement) of polymer rings (13). He argues that this condensed structure can significantly reduce the equilibrium linking number (entanglement) of the rings. Our results could be interpreted in this language, but the observed nucleoid structure is ordered to such an extent that it is better represented as a filament than a folded polymer.

**Estimate of chromosome fiber width.** To estimate the width of the chromosome fiber, consisting of a linear stack of 10 kb plectonemes, we estimate the size of a single plectonemal loop. We treat the plectonemal loop as a single microfiber consisting of a duplex of double-stranded DNA molecules, with approximately twice the persistence length of double stranded DNA and half the length. The RMS end-to-end distance of the microfiber is:

\[
R \approx \sqrt{hL} \approx 0.6 \mu\text{m}. \quad [S33]
\]

suggesting that a chromosome fiber consisting of 10 kb plectonemal loops would have roughly the same width as the observed nucleoid filament.

**Lateral confinement leads to segregation forces.** Please see the discussion in the caption of Fig. S9.

**Elastic constant need not be mechanical in nature.** In this paper, we motivate the use of a fluctuating spring model by an appeal to the polymeric structure of the nucleoid. The resulting model is both tractable and predictive. But a number of the fundamental assumptions in this model are flawed. In short, we do not show that the polymer is in thermal equilibrium and the observed fluctuations are thermal in origin. In fact, since the chromosome is subject to remodeling by a number of processes that are clearly active (replication, transcription, etc), it is unclear how this assumption could be rigorously correct. Furthermore, it appears that at least some of the variation observed in our experiments is cell-to-cell variation and are not equivalent to the fluctuations of a single cell. This disordered is therefore quenched rather than thermal or even dynamic. On the other hand, the predictions of fluctuating spring models are almost certainly more generally applicable than the motivation of this model might suggest.

For instance, one of the most subtle assumptions in our model is the dependence of the intranucleoidal interactions on contour length: \( k_{12} = \gamma / \Delta L_{12} \). But this characteristic scaling is not the result of polymer or spring models, but instead a consequence of the central limit theorem. We therefore expect that this result will be quite generally applicable. The crucial assumption is that the polymer is linearly arranged with only local interactions. Each additional monomer adds a random contribution \( \Delta k \) to the positioning (whether thermal or quenched):

*Topological domains are thought to imply large, stable DNA loops which prevent the propagation of twist (S12).*
where $\Delta X$ is the mean displacement per monomer. The variance in the inter monomer positioning is then

$$k_{ij}^{-1} = \langle \Delta X^2 \rangle = (i+1-j) \langle \Delta x^2 \rangle$$

$$= \Delta L_{ij} \langle \Delta x^2 \rangle \sigma^2,$$

$$= \frac{\Delta L_{ij}}{\sigma^2}$$

where $\sigma^2$ is the contour length of a monomer. The only assumption we have made is that there are no long-range correlations in the random variable $\Delta x$. (Short range correlations only lead to a renormalization of the definition of a monomer.) Therefore, we expect quenched disorder to be described by an analogous mathematical model, although the interpretation of the spring constants is not mechanical.

Open questions.

The crossing region.

Although we have characterized the crossing region, the mechanism by which this low-packing-density region is maintained remains mysterious. A polymer model of a homogeneous polymer would not predict such a low-packing-density region. It is possible that this region is microscopically structurally distinct. For instance, it is known that the chromosome structural protein MatP bind 23 times in this region and the protein appears to have a role in chromosome condensation (S14, S15). On the other hand, this low-packing density could be a consequence of the kinetics of chromosome replication: The terminus region is last to be replicated. If the chromosome arms are packed into a chromosome fiber sequentially, this process would stall at the terminus since both poles are pulling the remaining short region in opposite directions. The location where the replication forks meet is known to be stochastic, which may explain the stochasticity of the genomic position of the crossing region.

A modeling subtlety is introduced by the stochastic position of the crossing region. Our model assumes that the identity of the sequence at the pole of the nucleoid is always the same. In fact this sequence varies cell to cell and this effect would tend to further disguise the confinement-induced minimum in the single-locus variance at the poles of the nucleoid and also potentially introduces other complications.

Three dimensions versus one.

Another subtlety involves the three-dimensional structure of the nucleoid. We have modeled the structure along the long axis of the cell only. If the nucleoid filament is flexible, the elastic modulus that we have described may in fact describe the filament itself bending rather than compressing in response to confinement.

Single-cell versus cell-to-cell variation.

We have characterized the nucleoid structure by using the snapshot or population technique, rather than by measuring structure by time lapse in single cells. The variation we measure is therefore a combination of the dynamic stochastic fluctuations of the nucleoid structure in single cells and the cell-to-cell variation of this structure. A key follow-up to the analysis we present in this paper will be the determination of whether the observed variation is dominated by temporal fluctuations or cell-to-cell variation. If the observed variance is dominated by cell-to-cell variation, this will suggest a kinetic freezing model of nucleoid structure that will have a profound influence on the interpretation of the structural variation. In fact, experiments have already demonstrated that the delay of the replication fork on one arm of the chromosome can lead to structural changes, arguing that the kinetics of segregation play an indispensable role in determining (and therefore understanding) nucleoid structure (S16).

Fig. S1. (A) The fusion expression system pAW34. The fluorescent fusions, mCherry-lacI, tetR-EYFP, and CFP-parB, are inducibly expressed from the plasmid pAW34. (B) parS insertion plasmid. The suicide plasmid pJSM2048 can only be replicated by strains expressing the pir gene. In addition to the transposon flanked by inverted repeats (IR), the plasmid carries; an ampicillin resistance, an origin of transfer (oriT), and the transposase gene. When the transposon jumps into the genome of the recipient strain (that cannot replicate the suicide plasmid), the ampicillin resistance is lost, but the chlorphenocol resistance gene is integrated into the genome.

Fig. S2. The distribution in cell length for cells with a single detectable copy of the labeled loci. For the cell size analysis discussed in The dependence of the model parameters on cell length, we subdivided this population into Small (S), Medium (M) and Large (L) cells as shown in the figure.

Fig. S3. Quantifying the position distributions of ter-proximate loci. ter-proximate loci are poorly approximated by a single Gaussian distribution. The distribution of the ter-proximate loci in IL06 (white points) is fit by the ter fit function (black line). The fit function has two positioned states on either side of the locus (represented by Gaussian distributions, dotted) with independent positions, variances, and amplitudes. In addition, an unpositioned distribution, representing the crossing region (dashed line), allows loci to occupy the body of the nucleoid between poles.
**Fig. S4.** (A) The positioning distributions of ter-proximate loci. As the probe locus moves down the left arm of the chromosome towards ter (ter5 → B8 → ter6), the most probable locus position moves towards the pole of the nucleoid, and the distribution exhibits a strong asymmetry (a steep decay on the pole-proximate side of the distribution and a very long tail on the midcell side of the distribution). At ter6, the locus can be positioned at either pole, or unpositioned between poles. As we move up the right arm of the chromosome, we lose the positioned state on the left-hand-side of the nucleoid (D9). (B) The crossing probability. The probability of being in the unpositioned crossing region is plotted as a function of genomic position. The structural significance of this unpositioned state is clear from the inset. A low-packing-density “fiber” connects the two poles of the nucleoid.

**Fig. S5.** Intranucleoid interactions are not unique to oriC. The plot above shows the variance of the interlocus distribution as a function of the magnitude of the interlocus distance for two sets of locus pairs in IL01t (oriC-probe and probe-lac) and one set in IL05 (oriC-probe). The ± label denotes the genomic order of the loci.
Fig. S6. Fluctuating Spring Model of the Nucleoid. In the Fluctuating Spring Model, interactions are represented by fluctuating springs. The nucleoid is modeled as a fluctuating elastic filament with constant linear DNA packing density and elasticity \( \gamma \). In the expository model shown above, two loci are externally positioned: oriC and the left side of ter at the left pole of the nucleoid. The nucleoid model predicts that loci that are externally positioned are minima (cyan asterisks) in the variance of locus positioning (cyan curve). Confinement of the nucleoid between positions \( X_- \) and \( X_+ \) acts to weakly position the right lobe of the nucleoid. The interlocus variance (green) grows linearly with interlocus genomic distance for closely spaced loci (green asterisk). This linear dependence is preserved as long as the loci are positioned relative to the same externally positioned locus. In the example above, the interlocus variance saturates at the left pole of the nucleoid where positioning is dominated by external positioning at ter.
Fig. S7. The Fluctuating Spring Model. (A) A schematic drawing of the Fluctuating Spring Model. In the nucleoid structure illustrated above, the nucleoid is positioned in the cell by confinement and intranucleoidal interactions only. The stretch modulus of the nucleoid is $\gamma$ and the loci are coupled to this backbone by plectonemic springs with stiffness $k_P$. The nucleoid is confined in a box length $X_{conf}$. (B) Confinement-induced nucleoid positioning. The effect of confinement on the single-locus variance can be subtle. Strong confinement leads to a well defined minima at the poles of the nucleoid, similar to those predicted by strong tethering, but weak confinement leads to very shallow features in the single-locus variance. (C) The fit of the observed single-locus and interlocus variance to the Fluctuating Spring Model. The observed single-locus variance data (points) and fit (dotted curve) are plotted in blue. The observed oriC-probe interlocus variance data (points) and fit (dotted curve) are plotted in red. The observed lac-probe interlocus variance data (points) and fit (dotted curve) are plotted in green. (D) Confinement-induced positioning predicts the fine-scale structure of the observed single-locus distributions. The predicted single-locus distributions (solid lines) are compared to experimental distributions (dots, shaded regions represent counting error) for oriC, C10 and A11 probe loci.
**Fig. S8.** Cell length dependence. (A) The single-locus variance as a function of genomic position by cell size. Small (S) and Medium (M) cells have a smaller variance and are therefore more precisely positioned than large cells (L). (B) The inter-locus variance as a function of genomic position by cell size. As the cell length increases, the variance (relative to the cell length) decreases and loci are therefore more precisely positioned (with respect to the nucleoid) in larger cells (relative to cell length). (C) The nucleoid stiffness as a function of cell length. The data points represent the nucleoid stiffness fit from the Fluctuating Spring Model for the Small, Medium, and Large bins. (The box height corresponds to the error in determining the stiffness and the box width corresponds to the variance in cell length.) The dotted line represents the cell length scaling predicted by the confinement model of positioning. (D) The strength of confinement as a function of cell length. The data points represent the strength of confinement fit from the Fluctuating Spring Model for the Small, Medium, and Large bins. (The box height corresponds to the error in determining the strength of confinement and the box width corresponds to the variance in cell length.) The dotted line represents the cell length scaling predicted by the confinement model of positioning. The confinement-induced positioning model captures the trend of length dependence of both the nucleoid stiffness and the strength of confinement.
Fig. S9. (A) Condensation occurs soon after segregation. Supercoiling and nucleoid associated proteins (orange) likely act shortly after replication (blue locus) to condense DNA into the observed nucleoid filament. (B–E) Excluded volume and confinement drives segregation and faithful positioning of a nucleoid filament. (oriC is represented by the red locus and ter by the yellow locus.) Laterally confined filaments feel a force (blue arrows) due to the excluded volume of the filaments themselves which acts to segregate the filament longitudinally (B). The ter (yellow locus) is anchored at the septum until shortly before cell division, which constrains the chromosome conformation (C). After the release of ter, the chromosome reorganizes. Confinement and excluded volume again induce forces (blue arrows) which act to align the filament with the longitudinal axis of the cell (D–E). The packing density (nucleoid length relative to the cell length) is crucial in this process. Too low a packing density results in no positioning since the length of the nucleoid filament is too long for a driving force to align it in the cell, as shown in (E, top). Tightly confined nucleoid filaments show precise cellular positioning (E, center), while nucleoid filaments short compared to the length of the cell exhibit poor cellular positioning.

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Table S1. Strain probe position table. The insert name refers to the well identifier.