





Nucleosomes play a dual role in regulating transcription dynamics

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Transcription has a mechanical component, as the translocation of the transcription machinery or RNA polymerase (RNAP) on DNA or chromatin is dynamically coupled to the chromatin torsion. This posits chromatin mechanics as a possible regulator of eukaryotic transcription, however, the modes and mechanisms of this regulation are elusive. Here, we first take a statistical mechanics approach to model the torsional response of topology-constrained chromatin. Our model recapitulates the experimentally observed weaker torsional stiffness of chromatin compared to bare DNA and proposes structural transitions of nucleosomes into chirally distinct states as the driver of the contrasting torsional mechanics. Coupling chromatin mechanics with RNAP translocation in stochastic simulations, we reveal a complex interplay of DNA supercoiling and nucleosome dynamics in governing RNAP velocity. Nucleosomes play a dual role in controlling the transcription dynamics. The steric barrier aspect of nucleosomes in the gene body counteracts transcription via hindering RNAP motion, whereas the chiral transitions facilitate RNAP motion via driving a low restoring torque upon twisting the DNA. While nucleosomes with low dissociation rates are typically transcriptionally repressive, highly dynamic nucleosomes offer less of a steric barrier and enhance the transcription elongation dynamics of weakly transcribed genes via buffering DNA twist. We use the model to predict transcription-dependent levels of DNA supercoiling in segments of the budding yeast genome that are in accord with available experimental data. The model unveils a paradigm of DNA supercoiling-mediated interaction between genes and makes testable predictions that will guide experimental design.

supercoiling | nucleosomes | transcription

Supercoiling of the genomic DNA is a ubiquitous feature of active transcription in both eukaryotes and prokaryotes. Translocation of the RNA polymerase (RNAP), an active process generating RNA transcripts, overtwists the downstream DNA and undertwists the upstream DNA. First conceptualized in the twin-domain model more than three decades ago (1), the transcription-supercoiling interplay has come into renewed focus with recent experimental advances that allow tracking of individual transcribing RNAPs (2, 3) and genome-wide profiling of the DNA supercoiling (4, 5). Transcription-generated supercoiling has been shown to speed up transcription elongation via collective RNAP behavior (3), influence gene burst kinetics (2, 6, 7), and impact the three-dimensional genome architecture (8–11).

Theoretical and computational models of the transcription-supercoiling interplay have been immensely useful in interpreting experimental observations and making testable predictions to guide experimental design (12–16). These theoretical frameworks have to date focused on prokaryotic transcription and have accordingly incorporated the torsional response of bare DNA with varying levels of detail. However, the applicability of these models to eukaryotic transcription is unclear. This is because eukaryotic DNA predominantly resides in a nucleosome-wrapped state, termed chromatin, that is known to exhibit qualitatively different mechanics than bare DNA (17–19). While experimental studies are increasingly probing the role of the supercoiling in eukaryotic transcription (5–8), there lacks a theoretical framework that quantitatively analyzes the transcription-supercoiling interplay in chromatin.

Nucleosomes can affect transcription in multiple ways, both chemical and mechanical. Chemically, histones, the constituent proteins of nucleosomes, serve as substrates for various epigenetic modifications. These modifications can affect the recruitment of different components of the transcription machinery, as well as impact the three-dimensional genome architecture (20, 21). Mechanically, nucleosomes can serve as steric

Significance

DNA transcription by RNA Polymerase twists and supercoils the DNA. The resulting DNA torque mechanically restraints Polymerase motion, playing a regulatory role in transcription elongation. While this interplay of DNA twisting and Polymerase motion is well studied in bacteria, the applicability of the developed ideas to eukaryotes hinges upon understanding how nucleosomes modify the scenario. Here, we posit that nucleosomes may drive contrasting effects by coupling Polymerase motion with torsion in nucleosome-bound DNA or chromatin. Whereas nucleosome binding to the gene body may obstruct Polymerase motion, nucleosomes weaken chromatin torsional stiffness and thus reduce the mechanical constraints to eukaryotic transcription elongation. While model predictions for genome-wide supercoiling density agree with experimental data, the predicted changes upon transcription perturbation remain unverified.

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barriers to RNAP recruitment and translocation (22). Additionally, single-molecule assays support the notion that nucleosomes alter the torsional response of bare DNA (17–19). The observations suggest nucleosomes can act as torsional buffers, capable of absorbing or screening DNA supercoiling. This effect has been phenomenologically incorporated into a model of the transcription-supercoiling interplay (15). However, the absence of a quantitative model capable of predicting chromatin torsional response has held back a mechanistic treatment of supercoiling dynamics during eukaryotic transcription.

In this manuscript, we present a mechanistic framework to understand the role of DNA mechanics in eukaryotic transcription and its regulation, that is inspired by our previous work on prokaryotic transcription (14). Within this framework, transcription initiation is simulated as a stochastic event where RNAPs are recruited at the transcription start site (TSS) at a rate that sets the effective transcription initiation rate. Transcription elongation along topology-constrained (or net linking-number constrained) linear DNA, featuring translocation of the recruited RNAP and the associated transcription bubble, forces “arm wrestling” between the RNAP and the DNA. This is because the failure of a transcribing RNAP to rotate in congruence with the DNA groove results in an increased (reduced) DNA linking number density downstream (upstream). We simulate the DNA-twist-coupled translocation of RNAP via a set of dynamical equations that enforce torque balance between RNAP rotation and DNA twisting. This leads to increased rotation for less bulky RNAPs, while the DNA is twisted more when the RNAP bulk increases due to its attachment to larger mRNAs. The contest between RNAP rotation and DNA torsion is similar for prokaryotes and eukaryotes (14). However, within our approach, eukaryotic transcription is distinct because of nucleosome binding.

Nucleosomes play a dual role within this framework. Nucleosomes are modeled as structural units that exhibit stochastic binding dynamics. Bound nucleosomes may transition between chirally distinct conformations to minimize the elastic energy of the chromatin fiber. Simulating transcription along with stochastic nucleosome binding in topologically isolated genes, we find that while the nucleosomes binding the gene body act as steric barriers, the nucleosomes in the intergenic regions aid RNAP translocation (Fig. 1). Nucleosome chiral transitions convert twist-linking numbers of DNA into writhe, thus reducing the DNA torque-mediated hindrance to RNAP translocation. Although our results are agnostic to the origin of the supercoiling barriers, we believe compaction and/or tethering of DNA segments may serve as effective barriers to supercoiling diffusion. The torsional response of nucleosome-bound DNA or chromatin used in this study is developed by integrating nucleosome structural aspects with the twistable worm-like chain properties of bare DNA (Fig. 2). The major finding that nucleosomes reduce the torsional stiffness of DNA is consistent with single-molecule experiments (Fig. 2) (17, 18). Analyzing RNAP-induced DNA supercoiling in a chromatin context, we found that nucleosome-mediated torque buffering reinforces genes with lower transcriptional activity (Fig. 3). On the other hand, the steric hindrance aspect of nucleosomes, dominant for slow binding kinetics, counteracts transcription elongation (Fig. 4).

The model was used to simulate transcription in various kilobase-scale segments of the yeast *Saccharomyces cerevisiae* genome (Fig. 5). Predictions of the supercoiling status of these segments are in agreement with the statistical trends in experimental data (Fig. 6). The simulated genes typically show a higher propensity for negative supercoiling in the

promoter-proximal region where the nucleosomes adopt a negatively writhed conformation. Meanwhile, the gene terminus is likely to be positively supercoiled, with the nucleosomes adopting a less-negatively writhed (positive) conformation (Fig. 5). We also discuss how gene knockdown or overexpression affects other genes in the neighborhood via altered levels of DNA supercoiling (Fig. 5). Overall, this work argues that DNA supercoiling is an unavoidable and important aspect of actively transcribed eukaryotic DNA with structural and functional consequences at multiple length scales.

1. Results

1.1. A Statistical Mechanical Model Incorporating Chromatin Topology, Mechanics, and Nucleosome Chiral Transitions. We model chromatin as a string of nucleosomes wherein each nucleosome is a structural unit that absorbs 60 nm (177 bp) of DNA (Fig. 2A). This framework models nucleosome-driven DNA compaction and leads to a lower end-to-end extension of chromatin compared to bare DNA (Fig. 2E). To probe the mechanics of chromatin we mimic the experimental setup of a single-molecule tweezers experiment, where chromatin is fixed at both ends, put under an extensile external force and a fixed rotation or linking number (Fig. 2A). The DNA in the chromatin can exist in stretched or plectonemic states and the nucleosomes can exhibit different chiral states (Fig. 2B). While the stretched DNA state is stabilized by the extensile force and contributes to higher DNA extension, the plectonemic state arises when the applied DNA twist is large enough to buckle the DNA into a helically wrapped plectonemic configuration that stores linking number in the form of writhe, thereby absorbing DNA twist (23, 24).

The different nucleosome chiral states are defined by their configuration geometry (Fig. 2B). Following previous studies (17, 25), we posit three topological, or chiral, states of nucleosomes: open, negative, and positive. These states store differing amounts of DNA writhe due to differences in the geometry of how the DNA linkers exit the nucleosome core. When the two DNA linkers do not overlap or cross each other, the nucleosome is in an open state (Fig. 2B). Each nucleosome in the open state stores DNA writhe of $Wr_o = -0.7$ which comes from the inner turn of the nucleosome. When the two linkers cross each other, there is an additional contribution to the total DNA writhe of the nucleosome. If the linker crossing has the same topological sense as the inner turn, the net writhe of the nucleosome is more negative, $Wr_n = -1.4$, and we label it as the negative state (Fig. 2B). In contrast, if the linker crossing has the opposite sense to the inner turn, we call it the positive state with a net DNA writhe of $Wr_p = -0.4$ (Fig. 2B). These states can interconvert by rotations about the dyad axis. Such variations in nucleosome structure have been observed in cryoelectron microscopy studies (26). These nucleosome states are otherwise considered identical, such as in terms of their DNA binding energy and DNA length absorption (*SI Appendix*, see Figs. S2 and S3 for cases where this assumption is relaxed). Overall, a chromatin configuration with a fixed number of nucleosomes in open (n_o), positive (n_p), and negative (n_n) states will have a total nucleosome writhe given by

$$Wr_{\text{nuc}} = n_n Wr_n + n_o Wr_o + n_p Wr_p, \quad [1]$$

where $n_n + n_o + n_p = N$ is the total number of nucleosomes. Note that the writhe values for these states were chosen based on previous studies (17, 19, 27).

We specify the overall chromatin state by simultaneously specifying details of the DNA and the nucleosome configurations.

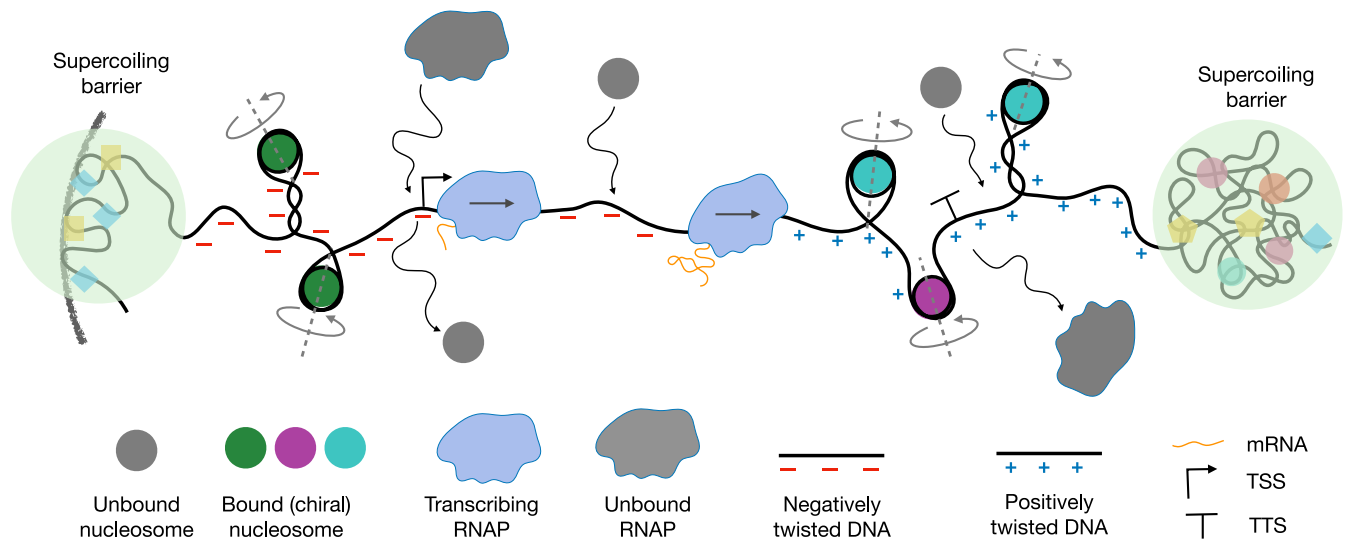


Fig. 1. Model for eukaryotic transcription. A topologically isolated eukaryotic gene with stochastic nucleosome binding. Transcription initiates via stochastic RNAP recruitment. RNAP translocation or transcription elongation proceeds as dictated by a mechanical torque balance between RNAP rotation and DNA twisting. Nucleosomes bound to the gene body hinder RNAPs. At the same time, bound nucleosomes rotate to undergo chiral transitions and absorb DNA torque generated by RNAP translocation, thus aiding transcription elongation. The flanking region upstream (downstream) of a gene is typically negatively (positively) supercoiled, whereas the gene body shows a gradient of negative to positive supercoiling. Compact or cross-linked DNA globules or segments of DNA tethered to nuclear bodies, like the lamina, are possible barriers to supercoiling in vivo.

The DNA configuration is specified by the DNA fractions in a force-extended or stretched state and a plectonemically buckled state. The nucleosome configuration is specified by the number of nucleosomes in each of the three chirally distinct states (Fig. 2 *A* and *B*). We write the total free energy for a given chromatin state:

$$F(\Delta Lk, f) = F_s(Lk_s, f) + F_p(Lk_p) + F_{nuc}(N), \quad [2]$$

where the RHS terms are the contributions from stretched DNA, plectonemic DNA, and nucleosomal states, respectively (*SI Appendix*, Eqs. **S1** and **S2**). ΔLk is the net change in the DNA linking number from a reference state; note that ΔLk is often described in an intensive form as the supercoiling density $\sigma \equiv \Delta Lk / (L_0 / h)$, where $h \approx 3.4$ nm is the length of the DNA double-helix repeat and L_0 is the total DNA length. Lk_s and Lk_p are contributions to the excess linking number from the stretched and plectonemic DNA states, respectively. The total free energy of the chromatin is minimized subject to the following linking number constraint:

$$\Delta Lk = Lk_s + Lk_p + \Delta W_{r_{nuc}}, \quad [3]$$

Here, $\Delta W_{r_{nuc}} \equiv W_{r_{nuc}} - W_{r_{ref}}$ is the deviation of nucleosomal writhe from the reference state $W_{r_{ref}}$. There are two possibilities for choosing $W_{r_{ref}}$. If a torsionally relaxed chromatin fiber is chosen as the reference, $W_{r_{ref}} = N W_{r_n}$ is an appropriate choice. This assumes that in the reference state, all the nucleosomes are in a negative state. Single-molecule experiments where torsional constraints are added after nucleosome assembly (18), as well as the in vivo scenario, correspond to this choice of reference (Fig. 2 *F* and *G*). The other possibility is choosing the relaxed, bare DNA as the reference, i.e., $W_{r_{ref}} = 0$. Single-molecule experiments where nucleosomes are assembled on torsionally constrained DNA (17) correspond to this choice. In such a scenario, the zero excess linking number state, which corresponds to relaxed bare DNA, has positively twisted DNA after nucleosome assembly (17) (Fig. 2*E*). Note that either choice of $W_{r_{ref}}$ ensures that $\Delta Lk = 0$ in the reference state.

Finally, for a given excess linking number ΔLk and extensile force f , we construct a partition function (24):

$$\mathcal{Z} = \sum_{L_p=0}^{L_0 - N\ell} \sum_{n_n=0}^N \sum_{n_o=0}^N \sum_{n_p=0}^N e^{-\beta F} \Theta(N - n_n - n_o - n_p), \quad [4]$$

where $\Theta(N - n_n - n_o - n_p) = 1$ if $n_n + n_o + n_p = N$, 0 otherwise. The torque follows:

$$\tau(\Delta Lk, f) = -\frac{1}{2\pi} \frac{\partial \ln \mathcal{Z}}{\partial \Delta Lk}. \quad [5]$$

Other observables, like the end-to-end extension (*SI Appendix*, Eqs. **S3** and **S4**) may be similarly obtained.

1.2. Chromatin Extension Is Less Sensitive to Excess Twist Than Bare DNA Due to Nucleosome Chiral Transitions.

When bare DNA is twisted, its extension initially remains unchanged since the force-extended state is stable in this regime. Beyond a threshold of excess twist, the DNA undergoes plectonemic buckling to compensate for the increasing DNA torque. Coexistence with the plectoneme state exhibits lower DNA extension as plectonemes do not contribute to extension (23, 24). This behavior is shown by the $N = 0$ curve in Fig. 2*F*. Modeling the chromatin twist response using the statistical mechanical model described above, we observe a qualitatively similar trend in the chromatin end-to-end extension as the bare DNA case (Fig. 2 *C*, *E*, and *F*), i.e., a hat-shaped curve. There are, however, two key differences. First, untwisted chromatin has a lower extension than untwisted bare DNA. This is a consequence of the nucleosome-driven compaction of DNA: each nucleosome absorbs 60 nm of DNA that can no longer contribute to the end-to-end extension. Consistent with nucleosome-driven compaction, we observe that the end-to-end extension decreases with an increase in the number of nucleosomes (Fig. 2 *E* and *F*). Second, the top part of the hat-curve, i.e., the regime with flat end-to-end extension, is wider for chromatin as compared to bare DNA. Additionally, the stability

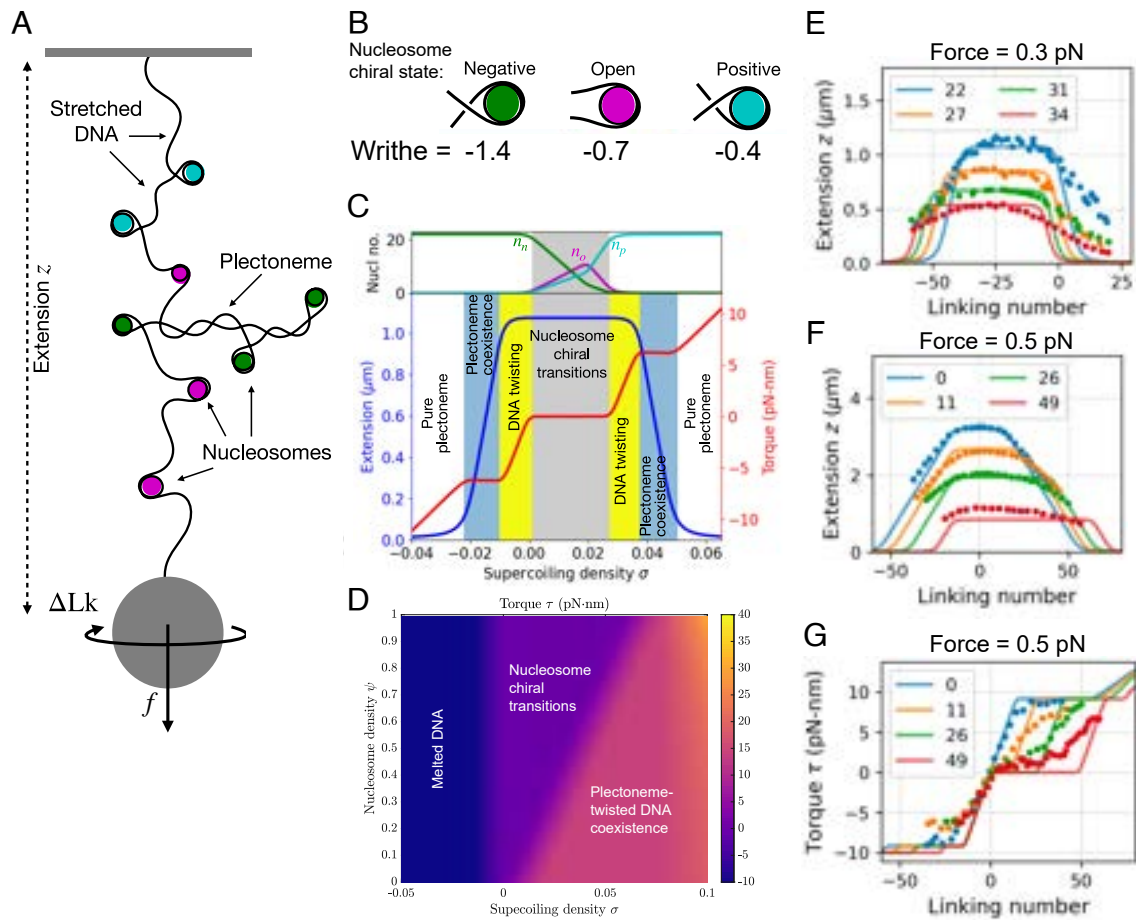


Fig. 2. Modeling the nucleosome-density-dependent torsional response of chromatin under a constant stretching force. (A) Schematic of the single-molecule tweezers setup commonly used to probe the torsional response of DNA/chromatin (17–19). The two ends of the chromatin segment are torsionally constrained, such that one is fixed to the surface of a coverslip and the other to the surface of a bead. The excess linking number in the chromatin segment ΔLk is controlled via the rotation of the bead. Additionally, the segment is put under a constant extensile force f . DNA in the chromatin fiber may wrap around nucleosomes, stretch under the external force, or buckle to form a plectoneme. (B) A DNA-bound nucleosome can exist in either a positive, open, or negative chiral state. These states store different amounts of linking numbers as writhe and may interconvert via simple rotation about the dyad axis, thus changing their writhe contribution to the linking number of the DNA segment. (C) Chromatin fiber extension (blue; left vertical axis) and torque (red; right vertical axis) as a function of the chromatin supercoiling density σ shown for a DNA segment of length 8.2 kb containing $N = 22$ nucleosomes, under a force of 0.3 pN, and using a reference state writhe $Wr_{ref} = NWr_n$. The distribution of nucleosomes among the different chiral states is shown in the *Top* panel. Note that injecting positive supercoils into the chromatin fiber leads to a flat regime in extension and a low torque valley, which is due to the coexistence of nucleosome chiral states. Beyond this valley, nucleosomes are unable to accommodate or buffer DNA twists leading to a chromatin response similar to that of bare DNA. The width of the low-torque valley increases with the number of nucleosomes, as a higher number of chiral transitions are able to buffer more DNA twists. (D) Chromatin extension z versus excess linking number ΔLk under $f = 0.3$ pN for a 8.2 kb DNA segment. The different colors correspond to different numbers of nucleosomes N as shown in the legend. Solid curves correspond to predictions from our model using $Wr_{ref} = 0$, while the dots indicate the experimental observations from Bancaud et al. (17). (E) Same as (D) for a 11.8 kb DNA segment under a 0.5 pN force, where $N = 0$ (blue) represents bare DNA. Note the shift in the x axis, which stems from using different reference writhes for the chromatin fibers in the two experiments (17, 18). (F) DNA restoring torque corresponding to the setup in E. Solid curves in E and F correspond to predictions from our model using $Wr_{ref} = NWr_n$, while the dots indicate the experimental observations from Le et al. (18). (G) Chromatin restoring torque for various supercoiling densities σ and nucleosome densities ψ at force $f = 1.0$ pN. Here, $\psi = 0$ corresponds to bare DNA and $\psi = 1.0$ corresponds to a chromatin fiber saturated with nucleosomes. This σ - ψ dependence of the restoring torque was used for all the simulations of chromatin transcription in Figs. 3–6.

of this regime, given by the width of the flat part, increases with an increase in the number of nucleosomes (Fig. 2 E and F).

When positive turns are injected into untwisted chromatin (the reference state with all nucleosomes in the negative state), the DNA does not twist in response. Instead, the nucleosomes undergo chiral transitions to a less negative state to accommodate the excess positive linking number. Negative nucleosomes first transition to the open states and then to the positive states (Fig. 2 C, *Top* panel). As there is no buckling, the chromatin end-to-end extension does not change in this regime. Once all the nucleosomes have transitioned to a positive state, any additional linking number can only be accommodated by DNA twisting that finally leads to buckling, i.e., plectoneme formation. Increasing positive turns further increases the fraction of plectonemic DNA that does

not contribute to extension leading to a steady decrease in end-to-end extension (Fig. 2 C, E, and F). Note that the open state is only transiently populated as shown in the top panel of Fig. 2C. However, introducing a lower DNA binding energy for the open nucleosome state, as has been argued (17, 25, 27), leads to a stable open state at interim supercoiling densities (*SI Appendix, Fig. S2*).

The stability of the unbuckled regime increases with the number of nucleosomes. This is because a larger number of chiral transitions allows for a DNA twist screening over a larger linking number range. As a result, the flat part of the “hat”-shaped regime increases with the number of nucleosomes (Fig. 2 E and F).

In contrast, when negative turns are injected into untwisted chromatin with all negative nucleosomes, the DNA immediately starts twisting since no nucleosome chiral transitions can ac-

commodate negative supercoiling in this scenario. As more negative twists are injected, the DNA buckles and starts forming plectonemes with negative writhe (Fig. 2C). Thus, chromatin's response to negative twists is the same as for bare DNA. Note that at higher extensile forces ($f \approx 1.0$ pN), the DNA may melt instead of forming negative plectonemes (23, 28). We model DNA melting assuming an effective torque threshold. When the DNA negative torque exceeds -10 pN·nm, there is a coexistence of melted DNA with twisted double-helix DNA leading to a torque plateau. Since we are mainly interested in the torsional response of the chromatin fiber, this approach is sufficient for this modeling.

Our predictions of the chromatin end-to-end extension in response to excess linking number injection are in agreement with the available data from two different single-molecule studies (17, 18) (Fig. 2E and F). Note that we did not do any parameter fitting in our model. Combining the previously calibrated worm-like chain model for double-helix DNA (23) with the nucleosome parameters (17) in a consistent framework was enough to get the quantitative agreement with experimental data (Fig. 2E–G).

1.3. Nucleosome Chiral Transitions Buffer DNA Restoring Torque. DNA twisting leads to a build-up of restoring DNA torque (23, 29). For bare DNA, the restoring torque increases linearly with the excess linking number. However, when the DNA torque is above a critical value, it is energetically favorable to buckle and pay the bending energy cost of a plectoneme instead of increasing the twist energy of unbuckled DNA. Once the DNA buckles into plectonemes, an increase in linking number is accommodated by an increase in plectoneme size and plectoneme writhe that keeps the DNA twist unchanged and the restoring torque plateaus (23). This behavior is shown by the $N = 0$ (blue) curve in Fig. 2G.

As discussed above (Section 1), in the case of chromatin, positive twists injected into the relaxed state (with all negative nucleosomes) are accommodated by nucleosome chiral transitions. Consequently, we obtain a regime with zero restoring torque for positively twisted chromatin (Fig. 2C and G). Consistent with the role of nucleosome chiral transitions in the emergence of this regime, the regime extends over larger ranges of positive supercoiling densities for higher nucleosome counts (Fig. 2D and G and *SI Appendix*, Fig. S7). The experimentally observed low torque valley near zero linking number is in accord with model predictions (18) (Fig. 2G). For the case of negative excess linking number and for positive excess linking number beyond the buckling threshold (i.e., once the DNA has started to form plectonemes), the chromatin restoring torque response is similar to bare DNA (Fig. 2G and *SI Appendix*, Fig. S7). Note that the key feature of the chromatin torsional response—a regime with near zero restoring torque for a range of positive supercoiling density—is robust to variations in the amount of writhe accommodated by the different nucleosome chiral states (*SI Appendix*, Figs. S4–S6).

Fig. 2D provides an overview of the chromatin restoring torque as a function of the supercoiling density σ and nucleosome density ψ . Here, we use a higher extensile force ($f \approx 1.0$ pN) which melts DNA at relatively lower negative supercoiling densities (23, 28). We computed the torque for a discrete set of ψ and σ values followed by linear interpolation to generate a smooth contour plot shown in Fig. 2D. This interpolation was then used to generate the restoring torque in the simulation of RNAP dynamics throughout this manuscript. The coexistence of melted and twisted DNA leads to a plateau in the negative

torque (*SI Appendix*, Fig. S7). A regime with a positive torque plateau is seen for positive supercoiling densities involving the coexistence of twisted and plectonemic DNA. While this regime is also seen both in bare DNA and in the case of chromatin, the onset of this regime in the case of chromatin occurs at higher values of σ for higher nucleosome densities. This effect is due to the ability of chiral nucleosome transitions to accommodate positive supercoiling thereby delaying DNA buckling. These two regimes of torque plateaus are in addition to the chiral-transition-driven regime of near-zero torque discussed earlier.

1.4. Coupling Chromatin Torsional Response with RNAP Dynamics to Model Eukaryotic Transcription. During transcription elongation, the RNAP must track the helical groove of the DNA, accumulating a rotational angle of $\omega_0 x$ when transcribing a DNA segment of length of x nm. Here, $\omega_0 \equiv 2\pi/b \approx 1.85 \text{ nm}^{-1}$ is the linking number density in unstressed double-stranded DNA. If the genomic segment under transcription is torsionally constrained, this accumulated angle is partitioned between the rotation of the RNAP θ (and the associated nascent RNA) and the DNA twist at the site of the RNAP ϕ :

$$\omega_0 x = \theta + \phi \quad [6]$$

The angle ϕ determines the excess linking number injected into the genomic DNA and, thus, the restoring torque applied by the DNA or chromatin. Following the approach in ref. 14, we write a torque balance equation for each RNAP:

$$\chi \frac{d\phi}{dt} = \eta x^\alpha \frac{d\theta}{dt} - (\tau_f - \tau_b) \quad [7]$$

Here, χ is the DNA twist mobility, η is the coefficient of friction, and α is an exponent that determines how fast the viscous drag on the complex containing RNAP and nascent RNA grows with an increase in the nascent RNA length (which equals x , the distance moved by the RNAP). The term ηx^α thus determines the rotational mobility of the RNAP. τ_f and τ_b are the restoring torques applied on the RNAP by the genomic segment downstream and upstream from the RNAP, respectively. While in the case of prokaryotes, τ_f and τ_b are only dependent on the excess linking number or supercoiling density in the respective genomic segments (i.e., $\tau_f \equiv \tau(\sigma_f)$ and $\tau_b \equiv \tau(\sigma_b)$), in the case of eukaryotes, the restoring torque will also depend on the nucleosome density in the genomic segments, i.e., $\tau_f \equiv \tau(\sigma_f, \psi_f)$ and $\tau_b \equiv \tau(\sigma_b, \psi_b)$ (Fig. 2D). The restoring torques applied by the chromatin segments on the RNAP were calculated using Eq. 5. Finally, the rate of RNAP translocation (dx/dt) is itself dependent on the net restoring torque acting on the RNAP with a torque-mediated stalling at $\tau_c = 12 \text{ pN} \cdot \text{nm}$ (14, 30):

$$\frac{dx}{dt} = \left(\frac{v_0}{2}\right) \left(1 - \tanh\left(\frac{\tau_f - \tau_b}{\tau_c}\right)\right), \quad [8]$$

where the maximum RNAP velocity is $v_0 = 60 \text{ bp} \cdot \text{s}^{-1}$. Eqs. 6–8 are solved to simulate the dynamics of a single RNAP.

To simulate transcription by multiple RNAPs, we consider a stochastic simulation setup wherein RNAPs are recruited to the TSS at a rate k_{on} (Fig. 3A). After recruitment, the dynamics of each RNAP is determined as described above. Supercoiling throughout the genomic segment is relaxed at a rate k_{relax} , mimicking the activity of enzymes such as topoisomerases. Nucleosomes stochastically bind and unbind from the genomic segment at rates k_{on}^{nucl} and k_{off}^{nucl} , respectively, independent of the supercoiling density in the genomic segment.

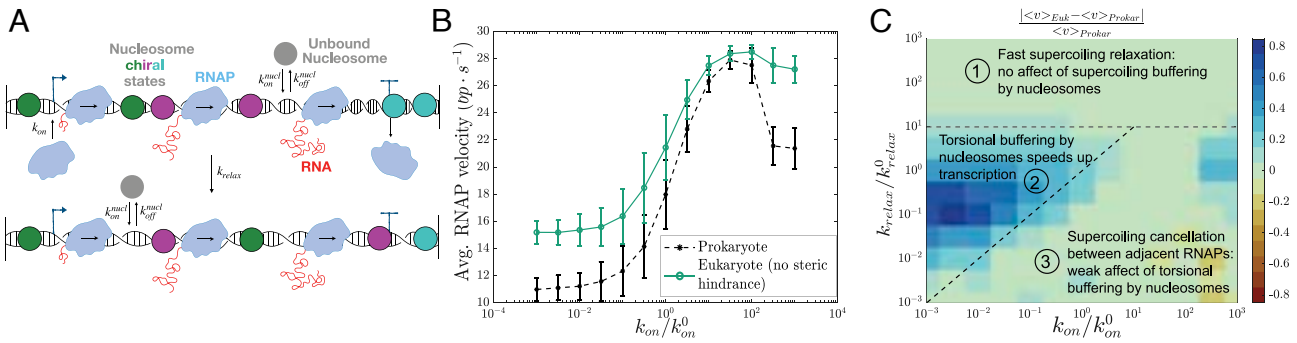


Fig. 3. Effect of nucleosome-mediated torsional buffering on transcription elongation kinetics. (A) A schematic of the model for supercoiling-coupled transcription in the presence of nucleosomes (i.e., eukaryotic transcription). RNAPs are recruited to the TSS at a rate k_{on} . At the same time, DNA supercoiling throughout the simulated genomic segment is relaxed at a rate k_{relax} , mimicking DNA topoisomerase activity. Nucleosomes can bind to specific sites on the genomic segment at a rate k_{on}^{nuc} and unbind at a rate k_{off}^{nuc} . The movement of each RNAP is coupled to the restoring torques applied by the genomic segments upstream and downstream (Eqs. 6–8), building upon an approach previously utilized to analyze prokaryotic transcription with bare DNA torque response (14). (B) The average RNAP velocity varies nonmonotonically with k_{on} in both prokaryotes (without nucleosomes) and eukaryotes (with nucleosomes that do not sterically hinder RNAP movement). The presence of nucleosomes makes eukaryotic transcription elongation faster at low and high k_{on} , while they are similar for intermediate values of k_{on} . Nucleosome-driven weakening of chromatin torsional stiffness underlies the RNAP speed up in eukaryotes. We estimate the RNAP velocity from the total time an RNAP takes to finish transcription. The error bars indicate the SD for the average velocity of each RNAP over 16 simulation trajectories. (C) Percentage change in the average RNAP velocity in eukaryotes as compared to prokaryotes for different values of k_{on} and k_{relax} . We indicate three distinct regimes. Regime 1: high topoisomerase activity where DNA torque-mediated constraints are minimal due to fast supercoiling relaxation, and hence torsional buffering by nucleosomes does not affect transcription kinetics. Regime 2: torsional buffering by nucleosomes significantly speeds up eukaryotic transcription for genes with a lower initiation rate and lower topoisomerase activity. Regime 3: collective RNAP behavior, featuring supercoiling cancellation between adjacent RNAPs. Nucleosome-mediated torsional buffering has little effect in this regime since supercoiling-mediated RNAP slowdown is already being mitigated by their collective behavior. Here, we used a gene of 5.3 kb with 10 kb flanking regions on each side, and the rate constants are $k_{on}^0 = 0.5 \text{ min}^{-1}$ and $k_{relax}^0 = 5.0 \text{ min}^{-1}$.

1.5. Nucleosome-Mediated Torsional Buffering Speeds Up Transcription Elongation. We used the above-described setup to simulate the transcription of a 5.3 kb gene (Fig. 3 and *SI Appendix, Fig. S8*). We begin with the assumption that nucleosomes do not present any steric hindrance to RNAP movement, supported by previous studies reporting cooperative interactions between subunits of the RNAP complex and nucleosomes that can facilitate transcription through nucleosomes (31). We find that the average rate of transcription elongation, given by the RNAP velocity, varies nonmonotonically with the rate of transcription initiation k_{on} (Fig. 3B). The increase of RNAP velocity with an increased rate of initiation originates from the cancellation of supercoiling between adjacent RNAPs. This regime of collective RNAP behavior has been observed experimentally (3) and in our previous model of prokaryotic transcription (14), i.e., without nucleosomes. We find that the collective regime is not much perturbed by the presence of nucleosomes, rather, the low initiation regime is significantly affected (Fig. 3B).

At low k_{on} , on average, a single RNAP is transcribing the gene at any given time. In this regime, the transcription elongation rate in eukaryotes is higher since the presence of nucleosomes lowers the net restoring torque acting on the RNAP as compared to the prokaryotic case of bare DNA (Fig. 3B). At higher k_{on} , multiple RNAPs transcribe the gene simultaneously at any given time (*SI Appendix, Fig. S10*). We find that an RNAP transcribes faster if additional RNAPs are subsequently recruited to the TSS behind it (*SI Appendix, Fig. S9*), which originates from supercoiling cancellation and lies at the crux of the RNAP cooperation. Supercoiling cancellation in this regime diminishes the effect of DNA or chromatin torsional response on transcription; consequently, the difference between prokaryotic and eukaryotic average RNAP velocities decreases, and the two approach one another (Fig. 3B). Finally, at very high k_{on} , we obtain a “traffic jam”-like regime, where the average RNAP velocity is likely to be determined by the translocation rate of the most downstream RNAP (14). The translocation rate of this RNAP will depend on

the restoring torque applied by the DNA or chromatin segment downstream from the gene body. The lower restoring torque in the case of chromatin underlies the higher average RNAP velocity of eukaryotes in this regime (Fig. 3B).

In addition to k_{on} , the average RNAP velocity is also dependent on the rate of supercoiling relaxation k_{relax} , where faster relaxation speeds up transcription elongation (3, 14). Comparing the average RNAP velocities in prokaryotes and eukaryotes in the k_{on} - k_{relax} space, we identified three regimes with distinct behaviors (Fig. 3C). At very high k_{relax} (regime 1), fast supercoiling relaxation makes DNA torque-dependent effects irrelevant and the RNAP velocities are the same in prokaryotic and eukaryotic cases. Similarly, in the regime of emergent collective behavior between cotranscribing RNAPs (regime 3), the RNAP velocities are similar for both prokaryotes and eukaryotes. Supercoiling cancellation between adjacent RNAPs makes the effects arising from the altered torsional response of chromatin less prominent. At low k_{on} and low k_{relax} (regime 2), the DNA or chromatin torsional response strongly influences RNAP translocation (Fig. 3C). Consequently, the average RNAP velocity is higher in the eukaryotic case with nucleosomes buffering the restoring torque acting on the RNAPs.

1.6. Steric Hindrance from Nucleosomes with Slow Turnover Impedes Transcription Elongation. We now investigate the role of steric interactions between nucleosomes and RNAPs on transcription elongation. Fig. 4 shows the behavior when nucleosomes act as rigid barriers to RNAP movement and an RNAP must wait for the nucleosome downstream to unbind before it can move forward. As expected, the average RNAP velocity is very low at low rates of nucleosome unbinding from the genomic DNA. The average RNAP velocities at all k_{on} values increase for faster nucleosome unbinding, approaching the scenario with no steric hindrance at very fast nucleosome unbinding rates. Note that the emergent cooperation between cotranscribing RNAPs is present even with nucleosomes acting

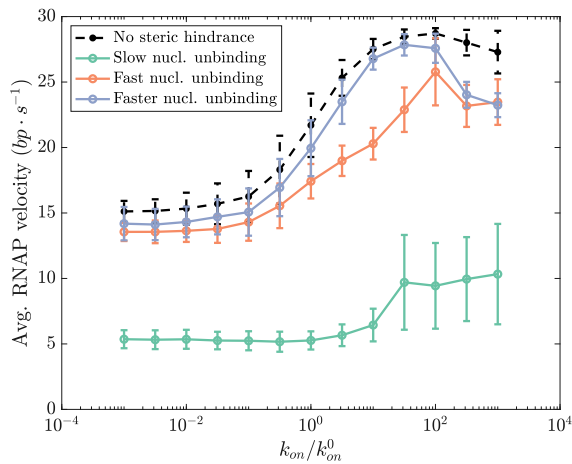


Fig. 4. Effect of steric hindrance from nucleosomes on transcription elongation kinetics. Average RNAP velocity as a function of the transcription initiation rate (k_{on}) for various nucleosome unbinding rates: slow ($k_{off}^{nucl} = 0.04 \text{ s}^{-1}$), fast ($k_{off}^{nucl} = 0.4 \text{ s}^{-1}$), and faster ($k_{off}^{nucl} = 4.0 \text{ s}^{-1}$). The nucleosome binding rate is kept unchanged in each case: $k_{on}^{nucl} = 1.2 \text{ s}^{-1}$. The dashed black line shows the case with no nucleosomal steric hindrance for comparison. Increased effective steric hindrance due to the slower unbinding of nucleosomes from the genomic DNA can decrease the average transcription elongation rate in eukaryotes. Since an RNAP must wait for the nucleosome in front of it to unbind before moving forward, the average RNAP velocity is lower at lower nucleosome unbinding rates. At higher nucleosome unbinding rates, the effective steric hindrance is lower, leading to kinetic behavior that resembles the no steric hindrance case. The error bars are computed in the same way as in Fig. 3B.

as steric barriers. This highlights that the cooperation regime is a key feature of transcriptional kinetics and is seen across contexts (Figs. 3B and 4).

Note that the treatment of nucleosomes as impenetrable barriers to RNAP movement is an extreme case. Experimental studies have shown that nucleosomes may need to only partially unbind from the DNA for the RNAP to pass through (31). Thus, our results spanning the no steric hindrance to impenetrable barriers include the expected behavior *in vivo*.

1.7. Predicting the Transcription-Dependent Supercoiling Profile in the Yeast Genome. We next simulated the transcription-supercoiling dynamics in long, multigenic segments of the budding yeast (*S. cerevisiae*) genome. The simulated segments were randomly chosen and ranged between 7 kb and 25 kb containing 4 to 25 genes. The k_{on} for each gene was chosen based on the gene expression level in the RNA-seq dataset from Guo et al. (5). Fig. 5 shows the supercoiling density profiles over two multikilobase yeast genome segments as predicted by our model. The predicted supercoiling density profile is a function of the transcriptional state since the k_{on} rates for the various genes in the two segments are inputs to the model. Importantly, the density profile changes in response to perturbing the transcriptional state of one of the genes. Our model makes two testable predictions: first, the supercoiling density profile, and second, the change in the density profile upon perturbing the transcription (Fig. 5). Note that suppression (knockdown or KD) (Fig. 5A) or overexpression (OE) (Fig. 5B) of a gene can alter the supercoiling density profile not only in the neighborhood of the perturbed gene but also over large genomic neighborhoods.

1.8. Transcription-Generated Supercoiling as a Mediator of Intergene Interactions. We probed the extent to which supercoiling-mediated interactions between neighboring genes

can emerge in real genomic contexts. When a specific gene is perturbed, we find that the RNAP velocities of the neighboring genes are typically strongly affected (Fig. 5). For example, when EGD2 is knocked down, the average elongation rates of its immediate convergent (LNP1) and divergent (NVJ1) neighbors increase (note the shifts in the probability densities of RNAP velocities in Fig. 5). Interestingly, the effect of knocking down EGD2 is not only limited to its immediate neighbors: the average elongation rates for ERG9 and UTP9 (one gene away), and RIX1 (two genes away) change as well. However, not all genes one or two genes away are affected. This suggests that supercoiling-mediated effects may propagate through genes in a context-dependent manner. Our model can quantitatively predict supercoiling-dependent variations in the transcriptional kinetics of real gene clusters as well as synthetic constructs, such as a multigene plasmid (7, 32).

Experiments have shown, both in prokaryotes (3, 32) and eukaryotes (7), that transcription-generated supercoiling can affect the transcription kinetics of neighboring genes in a manner dependent on the relative orientation of the genes. We previously showed for prokaryotes that RNAPs transcribing neighboring genes oriented in tandem can cooperate, speeding up one another. In contrast, RNAPs cotranscribing genes in divergent and convergent orientations antagonize and slow one another down (14). Since the qualitative coupling of RNAP translocation and DNA supercoiling in eukaryotes is the same as in prokaryotes, the qualitative rules for supercoiling-dependent neighbor interactions remain unchanged: activation for tandem and suppression for divergent and convergent orientations (Fig. 5 and *SI Appendix*, Fig. S13). Note that we do not incorporate supercoiling-dependent variations in transcription initiation (15, 33), which is expected to make the gene interactions more nuanced and is left for future studies.

1.9. Gene Bodies Show a Gradient of Supercoiling Accumulation. Analyzing the genes within our simulated segments, we find that the variation of the supercoiling density along the gene body depends on the transcriptional state of the gene (Fig. 5E). In the case of weakly expressed genes, there is minimal accumulation of DNA supercoils in the gene body. In the case of strongly expressed genes, negative supercoiling accumulates close to the TSS. Interestingly, the supercoiling density becomes more negative as one moves into the gene body, indicating the presence of highly untwisted DNA in the promoter-proximal part of the gene body. The supercoiling density then gradually becomes less negative toward the middle of the gene bodies and, eventually, positive close to the transcription end site. Since transcription over longer genomic distances generates more supercoiling, the gene body supercoiling density profile is further dependent on the gene length: longer genes accumulate more negative supercoiling close to the TSS as well as more positive supercoiling close to the gene end (Fig. 5E).

1.10. Predicted Genome-Wide Supercoiling Densities Compare Favorably with GapR-seq Experiments. Guo et al. (5) developed GapR-seq, an assay for profiling the level of positive supercoiling genome-wide in both prokaryotes and eukaryotes. Applying this method to the budding yeast *S. cerevisiae*, the study showed that the positive supercoiling accumulation was transcription-dependent. We simulated the supercoiling profile for 32 randomly chosen yeast genomic segments and compared it with the GapR-seq signal from Guo et al. (5) (Fig. 6). Note that while our model predicts the actual DNA supercoiling density, GapR-seq

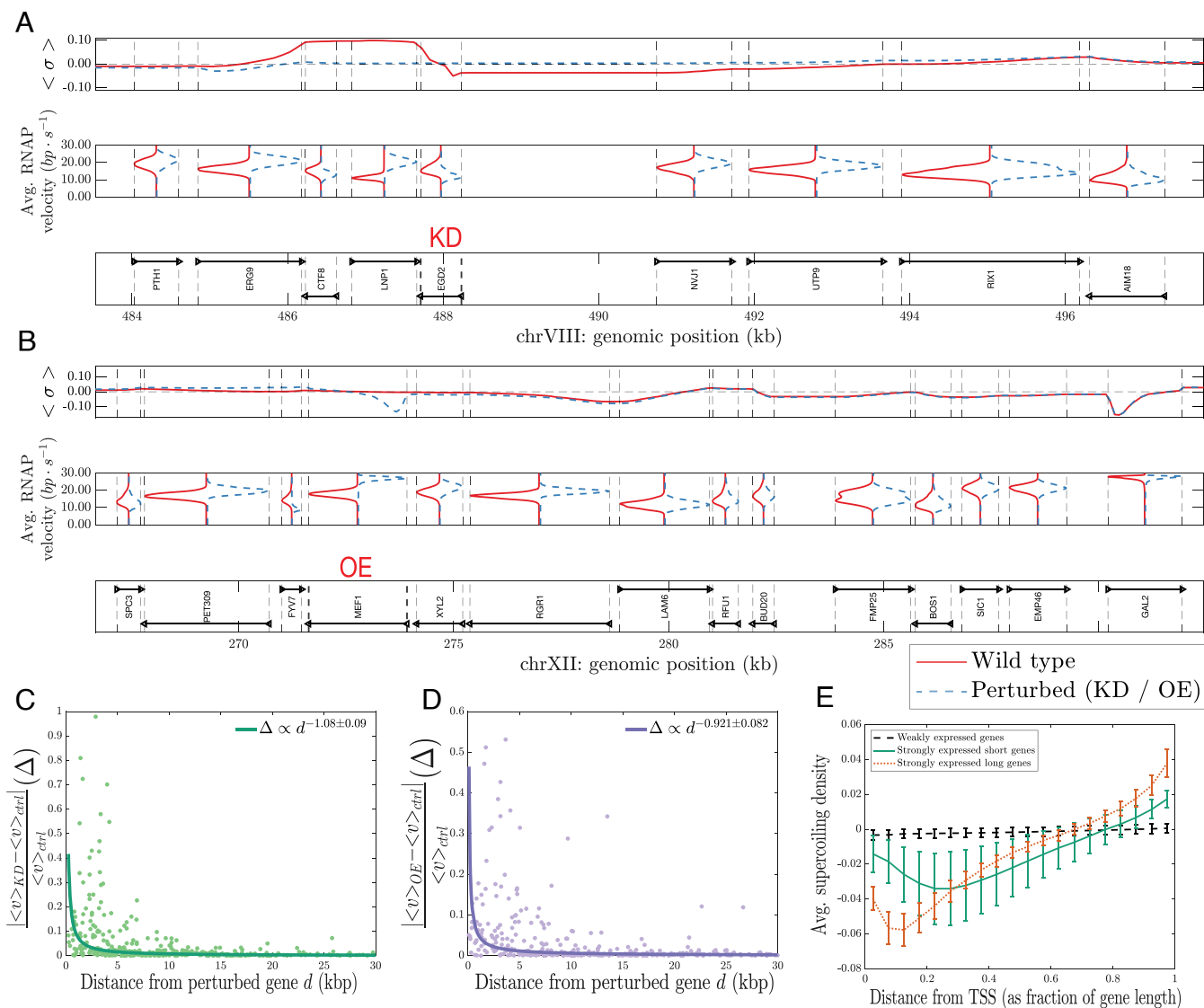


Fig. 5. Transcription-generated supercoiling can perturb the elongation kinetics of neighboring genes. Two representative yeast (*S. cerevisiae*) genomic segments are shown in (A) and (B). In each subplot, there are three panels. The *Top* panel shows the supercoiling density, the *Middle* panel shows violin plots with distributions of RNAP velocities for different genes, and the *Bottom* panel shows the gene annotations for the segment. Using the RNA-seq data from Guo et al. (5), we set the k_{on} for each gene (SI Appendix, section D). We used our model to predict the “Wild-type” supercoiling density profile and RNAP velocities for the genes in each segment (shown in red). We additionally show a “Perturbed” phenotype for the supercoiling density profile and the RNAP velocities corresponding to a scenario where one of the genes in the segment is perturbed (knockdown of EGD2 in A and overexpression of MEF1 in B, shown in blue). Overall, panels A and B illustrate the capability of our framework to model transcription-supercoiling interplay for real genomic segments that are tens of kilobases long and contain multiple genes. (C and D) Change in the average transcriptional elongation rate ($\langle v \rangle$) for genes as a function of the distance from the knocked down gene (C) or the overexpressed gene (D). As expected, the change in average elongation rate compared to the wild-type scenario decreases with the distance from the perturbed gene. The fit shown in each case was obtained by fitting a linear model to log-transformed data. (E) Model prediction of the average supercoiling density in the gene body of yeast genes with different lengths and expression levels. The average was calculated over 68 weakly expressed genes ($k_{on}/k_{on}^0 \leq 0.1$), 22 strongly expressed genes ($k_{on}/k_{on}^0 \geq 10.0$) shorter than 0.5 kb, and 21 strongly expressed genes longer than 1.5 kb. Genes were assigned k_{on} values based on the RNA-seq data from Guo et al. (5) (SI Appendix, section D). Error bars indicate the SEM.

assay reports the relative abundance of positive supercoiling at a genomic locus.

In agreement with the GapR-seq profiles, our simulations show that the extent of positive supercoiling is the highest in the intergenic regions between convergent genes and lowest in the regions between divergent genes (Fig. 6A and B). Analysis of the GapR-seq signal in gene bodies showed that there is no significant difference in the average GapR-signal near the start and end of gene bodies for weakly expressed genes (Fig. 6C). Whereas, for strongly expressed genes, positive supercoiling accumulated in the gene body close to the gene end (Fig. 6E). Both these trends were recapitulated for the supercoiling profiles predicted by our

model (Fig. 6D and F). Overall, our model simulations recapitulate transcription-associated supercoiling features obtained from genome-wide positive supercoiling profiling in yeast.

2. Discussion

In the present study, we have developed a mechanistic model for the transcription of eukaryotic DNA or chromatin (Fig. 1). The mechanical properties of chromatin are derived from the free-energy minimization of a twistable worm-like chain model (Fig. 2). Our model compares favorably with available experimental data and suggests chiral transitions by nucleosomes

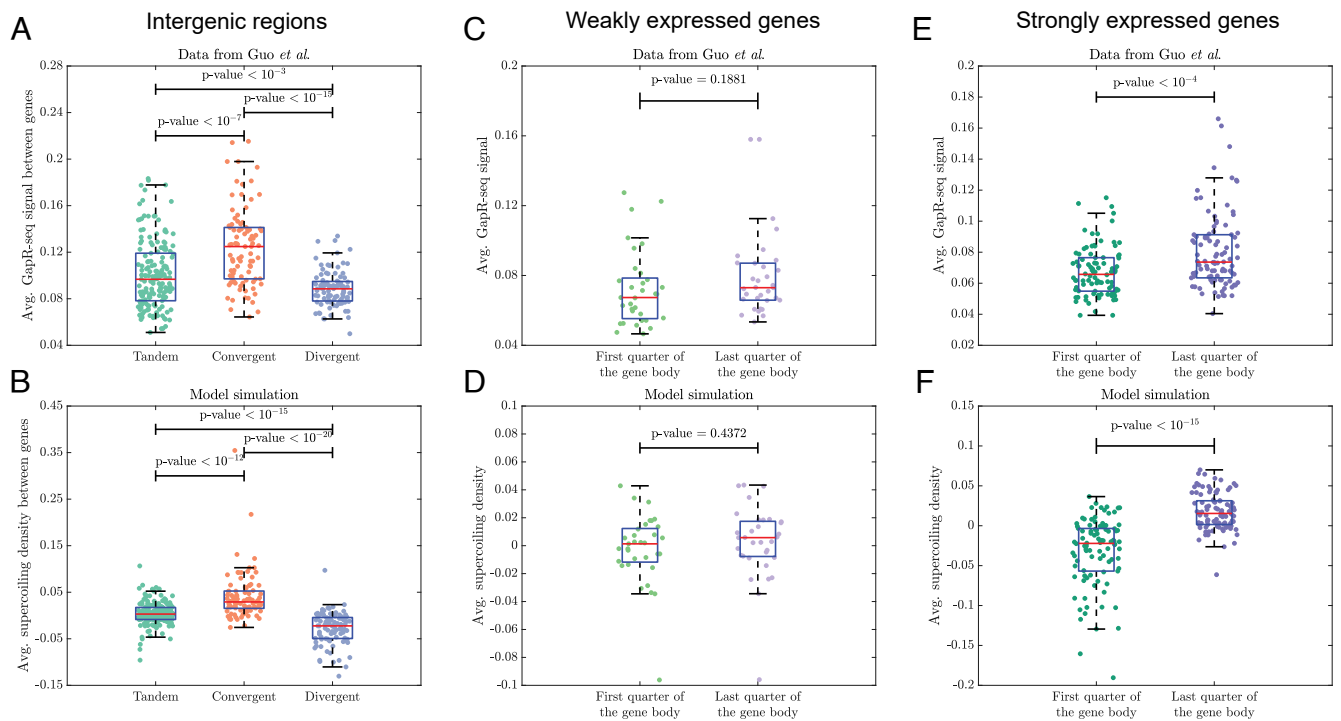


Fig. 6. Comparison of supercoiling densities predicted by model simulations with GapR-seq data for yeast. (A) GapR-seq (5) data for intergenic regions shows a higher signal for intergenic regions between convergent genes as compared to regions between divergent or in tandem gene pairs, indicating higher accumulation of positive supercoils in the regions between convergent genes. A total of 351 intergenic regions are shown: 170 regions between genes in tandem, 89 regions between convergent genes, and 92 regions between divergent genes. (B) Model simulations for yeast genomic segments containing the genes in A recapitulate the trend in supercoiling densities shown in A. (C) In the case of weakly expressed genes ($k_{on}/k_{on}^0 \leq 0.1$), the GapR-seq signal shows no significant difference between the beginning and end of the gene bodies. 33 weakly expressed genes are shown here. (D) Model predictions of supercoiling densities recapitulated the trend shown in C. (E) In the case of strongly expressed genes ($k_{on}/k_{on}^0 \geq 10.0$), the GapR-seq signal indicated a higher accumulation of positive supercoils close to the end of the gene body. 94 strongly expressed genes are shown here. (F) Model predictions of supercoiling densities recapitulated the trend shown in E. The transcription initiation rates for the genes in our simulations were chosen based on the RNA-seq data from Guo et al. (5) in the same manner as for Fig. 5. These k_{on} values were used to classify the genes as weakly or strongly expressed. Yeast GapR-seq profile (data shown in panels A–C) was taken from the study by Guo et al. (5). All P-values are for a two-sample *t*-test, with the null hypothesis that the data in the two groups are drawn from distributions with the same mean.

as the driver of the low torsional stiffness of the chromatin fiber (Fig. 2) (17, 18). These chiral states, storing differing amounts of DNA writhe, may interconvert via rotations about the dyad axis and accommodate DNA twists to weaken the torsional response (Fig. 2). We then integrated the chromatin torsion from this model into a previously proposed stochastic simulation framework (14) to investigate supercoiling-mediated aspects of transcription elongation kinetics in eukaryotes. Our major finding is that nucleosomes may have a dual effect on transcription. While binding to the gene body may hinder RNAP translocation, lowering the torsional stiffness of chromatin facilitates faster RNAP motion (Figs. 3 and 4). Using the model, we predicted the transcription-generated supercoiling profile in the yeast genome (Fig. 5). We find that genes may interact via DNA supercoiling, such that perturbation in the transcription state of a gene may significantly affect the RNAP motion for the genes in the neighborhood. Transcribed genes typically showed a negatively supercoiled TSS and a positively supercoiled transcription termination site (Figs. 1 and 5). We also found that the supercoiling accumulation in the intergenic regions depends on the relative orientation of the flanking genes (Fig. 5). Our results for supercoiling accumulation in the intergenic and genic regions are in agreement with the experimental observations (Fig. 6) (5).

Our model simulations show that, just as in the prokaryotic case, cotranscribing RNAPs in eukaryotes can cooperate to speed up transcription elongation (Fig. 3). Such supercoiling-mediated

cooperation, not requiring physical contact between adjacent RNAPs, has been experimentally confirmed in *Escherichia coli* (3). We predict that such a cooperative regime would be prominent in eukaryotes (Fig. 3B) as well, particularly under fast nucleosome turnover (Fig. 4). The gene orientation-dependent mechanical coupling of genes has also been observed both in prokaryotes and eukaryotes (3, 7). Our model, incorporating the complex interplay between stochastic RNAP recruitment, supercoiling dynamics, and gene orientation, can serve as a useful framework for analyzing the complex behavior seen in experimental studies, and for identifying physiological regimes of interest.

The statistical mechanical model used to calculate the chromatin torsional response in the present study is simple. Contributions from nucleosome stacking (19, 34) or DNA sequence dependence have been currently ignored. While the chiral transitions are central to the low torsional response, there may be a complex interplay between internucleosome interactions and chiral transitions, that is ignored in the present study. Note that the kinetics of these chiral transitions may also be influenced by epigenetic modifications on histone tails (35). Such considerations may be relevant to building more quantitatively accurate models as more experimental data become available. Another simplifying assumption employed in the model is the instantaneous relaxation of DNA twist and writhe, which allows using the equilibrium torsional response of chromatin fiber to obtain the restoring torque arising from linking number perturbations due to RNAP translocation. While twist relaxation

occurs at a very fast time scale, while relaxation requires the diffusion of bulky plectonemes and typically takes longer (36). Using an additional time scale associated with plectoneme diffusion within this framework, one can investigate its role in supercoiling accumulation and regulation of transcription. We reserve this as a future possibility. Furthermore, plectonemes in this model only regulate the DNA torque and offer no steric hindrance to the translocating RNAP. In principle, the presented framework could be extended to relax this constraint, however, we expect these effects not to affect the qualitative aspects of the result. In particular, plectonemes have been argued to form far away from the location of supercoil injection suggesting the steric aspects to not play a significant role (36).

Our model simulations can predict genomic supercoiling density profiles as a function of the transcriptional state (Fig. 5). The predicted supercoiling density profile may then be translated into predictions of nucleosomal conformations in different parts of the genome using our model of the chromatin torsional response (Fig. 2C and *SI Appendix*, Fig. S1). These predictions can be tested against nucleosome-level genomic structural features profiled by techniques such as Hi-CO (37) and RIC-seq (38). We note that such predictions would benefit from a more detailed model of the chromatin free energy (see ref. 39 for an example) such as one that incorporates higher-order chromatin structures (40, 41). We assume these segments (typically 10 to 20 kb long) to be insulated from a supercoiling perspective, which is in the same order of magnitude as bacterial supercoiled domains (42). A recent experimental technique, dubbed “Topo-Seq,” reports that the linking number of *in vivo* nucleosomes in Yeast follows a distribution centered around -1.26 and ranging from about -0.5 to -2.0 . This range is in line with the chiral states used in the model. Incorporating a larger number of chiral states to approximate the distributions is possible within this framework. Whether incorporating these states leads to better agreement with single-molecule experimental data (Fig. 2) remains to be seen.

It has long been recognized that nucleosomes present a steric barrier to transcription, both *in vitro* (43) and *in vivo* (44, 45). Our model of transcription elongation in eukaryotes shows that this inhibition is not the only mechanical effect of nucleosomes on transcription: nucleosomes can buffer RNAP-generated DNA torque and speed up transcription elongation. Thus, the overall effect of nucleosomes on the transcription elongation rate depends on the relative contribution from the two opposing effects (Fig. 4). Quantitative estimates concerning the nucleosomal barrier to RNAP movement are lacking. However, the fact that average transcription elongation rates in eukaryotes and prokaryotes are comparable would suggest that eukaryotic transcription operates in the regime of weak steric hindrance (or fast nucleosome unbinding; see Fig. 4). Multiple processes have been implicated in such modulation of the nucleosome barrier (22, 31). The presence of the histone variant H2A.Z (instead of H2A) in nucleosomes has been shown to increase the nucleosome turnover rate, reducing the barrier to transcription (46, 47). The histone chaperone FACT, which travels with the RNAP, can relieve RNAP stalling at nucleosomes by destabilizing histone–DNA contacts (48) and promoting nucleosome eviction (49). The various nucleosome remodelers, that use ATP to assemble, evict, or slide nucleosomes, also serve to alter the overall magnitude of the steric hindrance effect of nucleosomes on RNAPs (22). These mechanisms of attenuating the nucleosome steric hindrance, along with RNAP speed-up from torsional buffering by nucleosomes, ensure fast transcription in eukaryotes. The modeling framework can be used to predict

the qualitative effect of perturbing any of the aforementioned mechanisms.

Note that chromatin supercoiling can itself alter nucleosomal dynamics. Single-molecule assays have shown that nucleosome assembly is faster on negatively supercoiled DNA while positive supercoiling inhibits nucleosome binding (50). A similar assay has shown that positive supercoiling can evict H2A/H2B dimers from nucleosomes, leaving behind tetramers (51). Consistent with this observation, nucleosomes have been shown to be depleted from the region downstream of a highly transcribed gene in yeast (52). In the present study, with a focus on the effect of torsional buffering on transcription elongation, we have simulated the simpler scenario where the nucleosome binding/unbinding kinetics are independent of the supercoiling density. Additionally, in contrast to previous theoretical studies (15, 33), we have assumed that transcription initiation (i.e., the model parameter k_{on}) is not a function of the supercoiling density at the TSS. Both these dependencies may be incorporated into the approach described here and present promising future directions.

Comparing the predicted supercoiling density profiles in different genomic regions with the three-dimensional chromatin architecture of these regions obtained by Hi-C assays (53) is an exciting prospect. While it is not clear which elements constitute supercoiling barriers, three-dimensional structures like compact globules or chromatin segments attached to nuclear bodies like lamina may act as barriers to twist diffusion since DNA may be heavily cross-linked in these regions. Diffusion of supercoils by rotation of these barriers is also a possibility that may be incorporated in the model. Although a connection between chromatin supercoiling and 3D chromatin architecture has been posited (for example, see *SI Appendix*, Fig. S2J, and the accompanying discussion in ref. 54), conclusive studies are lacking due to technical challenges like low resolution of supercoiling density genome-wide (55) or the inability to profile both positive and negative supercoiling levels (5). Predicted transcription-dependent supercoiling profiles could help identify genomic regions where aspects of transcription, supercoiling, and 3D genome may be probed by targeted experiments (11). The present model could further be extended to include additional biological processes that have been shown to exhibit supercoiling dependence such as the formation of R-loops (56) and recruitment of SMC complexes (57, 58). Altogether, the model of supercoiling-transcription interplay described here can serve as a foundation for developing a DNA mechanics-based connection between genome architecture and cellular function.

Data, Materials, and Software Availability. Previously published data were used for this work ([GSM5001912](https://doi.org/10.1101/2019.08.01.264192) (59) and [GSM5001899](https://doi.org/10.1101/2019.08.01.264193) (60)).

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The authors declare no competing interest.

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