Mechanical Properties of Transcription

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The mechanical properties of transcription have recently been shown to play a central role in gene expression. However, a full physical characterization of this central biological process is lacking. In this letter we introduce a simple description of the basic physical elements of transcription where RNA elongation, RNA polymerase rotation and DNA supercoiling are coupled. The resulting framework describes the relative amount of RNA polymerase rotation and DNA supercoiling that occurs during RNA elongation. Asymptotic behavior is derived and can be used to experimentally extract unknown mechanical parameters of transcription. Mechanical limits to transcription are incorporated through the addition of a DNA supercoiling dependent RNA polymerase velocity. This addition can lead to transcriptional stalling and important implications for gene expression, chromatin structure and genome organization are discussed.

PACS numbers:

The helical nature of DNA introduces a physical dimension to many important biological processes. Most notably transcription, which is the first step in the conversion of genetic material into biological matter. Though the study of transcription has played a central role in modern molecular biology much of its physical foundation and behavior is just now being appreciated [1]. Characterizing the physical aspects of transcription may offer insights into many open problems in gene expression and biology.

The physical nature of transcription is conceptualized in the twin-domain model [2] where it was first articulated that transcription and replication cause over-twisting and under-twisting of DNA. The over or under twisting of DNA is referred to as supercoiling (SC) and a number of experimental observations have revealed its central role in transcription [3]. Recent results have pointed to SC and mechanical feedback as the source of transcriptional bursting [4] and domain formation in bacteria [5]. However, a full description of this phenomena is still lacking.

In this letter we will introduce a simplified description of transcription with the three fundamental coordinates of DNA rotation, RNA polymerase (RNAP) rotation and RNA elongation. Due to the helical nature of DNA, linear RNA elongation is coupled to rotational motion of both RNAP and connected nascent RNA. We will refer to RNAP and nascent RNA collectively as the RNA complex (RNAC). Naturally, the basic coordinates are the RNAC position along a particular gene from the transcription start site (TSS) x and the relative rotation of the RNAC $\theta(x)$ and the DNA $\phi(x)$. These quantities are tied together as

$$\omega_0 x = \phi(x) + \theta(x) \tag{1}$$

where $\omega_0 = 1.85 nm^{-1}$ encodes the natural linking number of DNA. The relative difficulty in twisting the DNA (because of opposing torque) or difficulty rotating the RNAC (because of drag) determines the form of the functions $\phi(x), \theta(x)$. The relative amount of DNA twisting ϕ and RNAC rotation θ can be determined by the balance between DNA torque $\tau(\phi)$ and RNAC drag $\Gamma(x,\dot{\theta})$ as

$$\tau(\phi) = \Gamma(x,\dot{\theta}) \tag{2}$$

While many mechanical properties of DNA are well characterized, the mechanical nature of the rotating RNAC is largely unknown. From early studies however it is clear that RNA elongation plays a key role [6, 7]. Even though this is a critical factor, the coefficient and functional dependence of transcript length on the rotational drag Γ are not known at this time. We will posit an RNAC viscous rotational drag which is linear in the rotation speed with a power-law dependence on the transcript length as $\Gamma = \eta x^\alpha \dot{\theta}$ where $\dot{\theta}$ is the angular speed of the RNAC and η an unknown coefficient of friction.
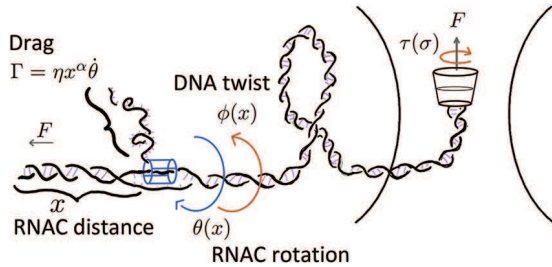

FIG. 1: (color online) A cartoon depicting RNA elongation x through shared RNAP θ and DNA ϕ rotation. The DNA is attached to an optical bead for mechanical manipulation.
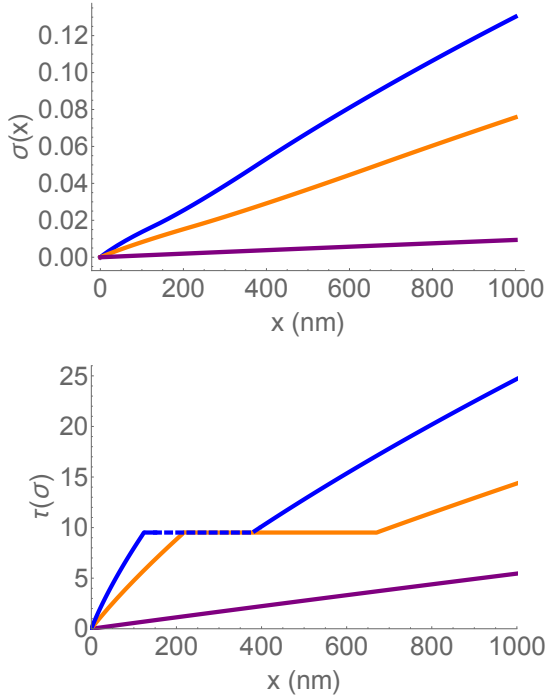
FIG. 2: (color online) Supercoiling σ and torque τ as a function of RNA elongation x for $L = 5, 10, 100\ \mu m$ (blue, orange, purple) with $\alpha = \frac{1}{2}$, $\eta v = 1$ at a fixed force of $F = 1/2$ pNnm ($S = 582.0$, $\tau_0 = 9.5$, $P = 189.6$ pnNm).

A length independent frictional term for the drag associated with the rotation of the RNAP can be included but is not used here considering the dominant role of elongation in generating SC [6, 7]. Previous studies have also linked elongating RNA structure to RNAC activity [8, 9]. Dotted and primed marks denote derivatives with respect to time and space respectively.

The mechanical properties of RNA polymerase itself are well characterized and it displays constant velocity [10] behavior over a wide range of torque (-20 to +12 pNnm) [11]. We will therefore start by assuming a constant elongation rate. Later we will introduce ways for incorporating the mechanical limits of the RNA polymerase into the motion of the RNA complex.

With an application of the chain rule we can turn the time derivative for RNAC rotation into a spatial derivative as $\dot{\theta} = \partial_t \theta(x(t)) = v\theta'$ where $v = \dot{x}(t)$ is the linear velocity of the RNAC. Using this identity with equations 1 and 2 we are left with an equation of motion for DNA twist as a function of RNAP translocation

$$x^\alpha \phi' + \frac{1}{\eta v}\tau(\phi) - \omega_0 x^\alpha = 0 \qquad (3)$$

We now imagine that there is an impediment to DNA twist a distance L ahead of the TSS. This can be done explicitly in an in vitro experiment (shown in Fig.1) or may occur naturally for DNA in vivo with obstructions or other actives areas of transcriptions. Doing

this turns the twist equation into an equation for supercoiling density $\sigma(x)$ (SCD) through the substitution $\sigma(x) = \frac{\phi}{\omega_0(L-x)}$; this expression assumes that twisting strain at the point of transcription immediately spreads throughout the specified DNA length (see below). At this time we will imagine only one barrier to DNA rotation ahead of the RNAC. These assumptions yield the equation

$$\omega_0 x^\alpha (L - x)\sigma' - \omega_0 x\sigma + \frac{1}{\eta v}\tau(\sigma) - \omega_0 x^\alpha = 0 \qquad (4)$$

Under the additional assumption that the length of the gene is much smaller than the distance to the obstruction we can drop the L^{-2} terms to find the SC equation

$$x^\alpha \sigma' + \frac{1}{\eta v \omega_0 L}\tau(\sigma) - \frac{x^\alpha}{L} = 0 \qquad (5)$$

To calculate the SCD $\sigma(x)$ as a function of translocation x using eq. 5 we must specify the torque response of DNA as a function of SC $\tau(\sigma)$. Supercoiling and DNA mechanical dynamics occur on a sub-second time-scale [12, 13] whereas typical speeds for transcription are $10 - 50\ \frac{bp}{s}$ [10]. This means that for genes on the order of 1 kbp transcriptional dynamics happen on the second and minute time-scales. Additionally, RNAP operation is robust against sub-second torque fluctuations [11]. Subsequently, as stated above, we expect the locally produced supercoiling at the boundary to spread throughout the allowed DNA segment on a time-scale faster than transcription occurs. More generally, we might expect to solve a supercoiling transport equation of the form

$$\frac{\partial \Phi(\tilde{x})}{\partial t} = D\frac{\partial^2 \Phi(\tilde{x})}{\partial \tilde{x}^2} \qquad (6)$$

with the boundary conditions for the local twist angle $\Phi = 0$ at $\tilde{x} = L$ and $\Phi = \phi(x)$ at $\tilde{x} = x$. The aforementioned limit appears if the effective rate of strain relaxation D is sufficiently fast, and this will be assumed in what follows. Finally, additional sources of SC dynamics will be ignored.

Thus the torque $\tau(\sigma)$ response will be that of steady-state supercoiled DNA over a length L. In this framework super-coiled DNA can exist in a purely twisted, purely plectonemic or a mixed state. Following the phenomenological approach given by Marko [14] the torque in a given piece of DNA held at a constant force is specified by the SCD as

$$\tau(\sigma) = \begin{cases} S\sigma, & \sigma < \sigma_s^* \\ \tau_0, & \sigma_s^* < \sigma < \sigma_p^* \\ P\sigma, & \sigma_p^* < \sigma \end{cases} \qquad (7)$$

where the coefficients S, τ_o, P and SC transition values σ_s^*, σ_p^* are given by DNA mechanical constants and are a function of applied force. It is worth noting that the introduction of a well-defined applied force is at this time
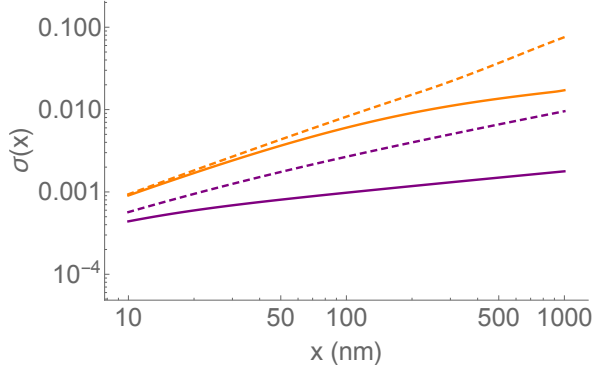
FIG. 3: (color online) Supercoiling σ as a function of RNA elongation x for $L = 10$ μm at a fixed force of $F = 1$ pNnm ($S = 622.6$, $\tau_0 = 14.5$, $P = 189.6$ pnNm). The solid and dashed curves are for $\alpha = \frac{1}{2}$ and $\alpha = \frac{1}{4}$ respectively and the colors are such that $\frac{\eta_{orange}}{\eta_{purple}} = 10$. The y intercepts are determined by the drag coefficient η and the slopes by the functional exponent α. The plot illustrates how the asymptotic behavior of SC and torque would allow for direct measurements of the unknown mechanical parameters of the RNAC.

cloudy from an in vivo perspective, its experimental implementation is straightforward.

This formulation of $\tau(\sigma)$ yields two types of equations for supercoiling during transcription. The first is for the constant torque response in the region $\sigma_s^* < \sigma_c < \sigma_p^*$

$$x^\alpha \sigma_c' + \frac{\tilde{\tau}_0}{L} - \frac{x^\alpha}{L} = 0 \qquad (8)$$

and the second for linear torque response in the two regimes $\sigma_l < \sigma_s^*$, $\sigma_p^* < \sigma_l$

$$x^\alpha \sigma_l' + \frac{\tilde{w}}{L}\sigma_l - \frac{x^\alpha}{L} = 0 \qquad (9)$$

where in both equations we have consolidated the constants as $\tilde{\tau}_0 = \frac{\tau_0}{\omega_0 \eta v}$ and $\tilde{w} = \frac{S}{\omega_0 \eta v}$, $\tilde{p} = \frac{P}{\omega_0 \eta v}$ respectively.

Analytical solutions are obtainable for both the constant and linear SC equations (eq.8 and eq.9 respectively). Numerical integration of the full nonlinear equation 4 (including L^{-2} terms) is possible but not considered here. For the constant torque equation (eq. 8) direct integration yields

$$\sigma_c(x) = -\frac{x}{L} + \frac{\tilde{\tau}_o}{L}\frac{x^{1-\alpha}}{1-\alpha} + K_1 \qquad (10)$$

which describes the SC as a function of RNAC elongation in the hybrid DNA response region. The constant K_1 is used to match SCD levels at the boundary.

The linear torque equation (eq.9) can be solved through the decomposition $\sigma(x) = f(x)g(x)$ with $g(x) = K_2 e^{-\frac{\tilde{w}}{L(1-\alpha)}x^{1-\alpha}}$. This leads to the solvable equation

$f' = \frac{1}{g}$ which can be used to obtain the full solution

$$\begin{aligned} \sigma_l(x) &= \frac{K_2}{L}e^{-\frac{\tilde{w}}{L(1-\alpha)}x^{1-\alpha}} \\ &+ \frac{1}{L}e^{-\frac{\tilde{w}}{L(1-\alpha)}x^{1-\alpha}}\int^x dy \; e^{+\frac{\tilde{w}}{L(1-\alpha)}y^{1-\alpha}} \end{aligned}$$

for the SCD as a function of RNAC elongation in the both the pure twist and pure plectonemic regions of DNA response. Again, K_2 is a constant used to match boundary conditions. Though the solution is in the form of an integral for arbitrary α, simple solutions exist for many rational values of α. Typical SCD results $\sigma(x)$ and corresponding torques $\tau(\sigma)$ are shown in Fig. 2 for several barrier distances. For both the pure twisting or pure plectonemic regime, where the SC is governed by eq.9 there is an important asymptotic response for the SCD solution σ_l in the limit $x^{\alpha-1} >> \frac{\tilde{p}}{L(1-\alpha)}$

$$\sigma_l \sim \frac{1}{\tilde{w}}x^\alpha \qquad (11)$$

so that in the asymptotic limit the SC and torque directly mirror the drag associated with RNAC rotation. This is an important result as it provides an easy method for measuring the unknown RNAC drag. Reaching the mixed $\sigma > \sigma_s^*$ and full plectonemic $\sigma > \sigma_p^*$ regimes occur when transcription lengths exceed the values of x_s^*, $\sigma(x_s^*) = \sigma_s^*$ and x_p^*, $\sigma(x_p^*) = \sigma_p^*$ respectively. Though a closed analytical expression for x_s^*, x_p^* is difficult, they can be numerically evaluated and for the case of x_s^* estimated through the asymptotic expression as $x_s^* \sim (\tilde{w}\sigma_s^*)^{1/\alpha}$.

Observations of the most basic elements presented in this letter can be obtained through laser trap experiments (shown schematically in Fig. 1). Independent determination of the drag coefficient η and the functional exponent α is in principle possible. This is shown in Fig. 3 where the slopes of $\sigma(x)$ on a log-log plot are determined by the value of the exponent α and the y intercepts through the value of the drag η.

We expect the functional dependence of the drag on RNA length to reflect the effective cross-sectional area of the nascent RNA. For a random polymer the effective cross-sectional area scales as the square root of the polymer length ($\alpha = \frac{1}{2}$), however higher order RNA structure and electrostatic interactions may drive the drag away from this behavior. Additionally, it is possible that the cross-sectional area depends on angular velocity which would generate higher level terms in eq. 5. While neglected here such effects could be incorporated into future models.

So far, we have assumed that RNAP moves at a constant velocity for all SC levels. However recent connections between the mechanical state of DNA, RNAC progress and transcriptional bursting have been brought to light both theoretically and experimentally [4, 15, 16].

These effects are rooted in the stalling torque of RNAP which we will call τ_c [11]. A natural way to implement this mechanical limit is through the incorporation of a torque dependent velocity

$$v(\sigma) = \frac{v_0}{1 + \left(\frac{\tau(\sigma)}{\tau_c}\right)^n} \tag{12}$$

yielding a modified equation eq.5 which now includes a stalling component

$$x^\alpha \sigma' + \frac{1}{\eta v_0 \omega_0 L}\left(\tau(\sigma) + \tau_c\left(\frac{\tau(\sigma)}{\tau_c}\right)^{n+1}\right) - \frac{x^\alpha}{L} = 0 \tag{13}$$

this new modified SCD equation can be numerically integrated to yield SC, torque and velocity curves as a function of elongation (Fig.4).

Additionally, the number of transcripts which can be made before $\tau(\sigma) > \tau_c$ for a gene of length W given a barrier a distance L is away is an important quantity for understanding the competition between mechanical frustration and relaxation in transcription [16]. To examine this we have integrated eq.5 for various W, L for a single RNAC operating on a gene and prohibited relaxation between independent transcriptional events. After a single RNAC has reached the end of the gene the resulting SC level is used as an initial condition for the subsequent integration of eq.5. The number of transcripts which can be made before $\tau(\sigma) > \tau_c$ is called m_c and it plays an important role in bounding transcriptional noise due to RNAP stalling [16]. The dependence of m_c on W, L is shown in Fig. 5.

It is thus clear that the physical location of a gene within the genome has a significant impact on its ability to be transcribed (Fig.4 and Fig. 5). The values shown are for convenient choices of the RNAC drag parameters and will not necessarily match real values. Experimental measurements of RNAC drag parameters (as outlined earlier in this letter) would allow for accurate predictions of the interplay between torque buildup and rotational drag. An enlarged framework which includes a stochastic competition between mechanical frustration (through transcription) and mechanical relaxation (through topoisomerase action) is left for future work and will shed further light on the in vivo properties gene expression.

The structural conformations realized by a particular piece of DNA is constrained by the SCD. The existence of domains in bacterial DNA has been linked to transcription and has been posited to be formed out of plectonemic DNA [5]. The framework presented here can be used to quantitatively predict the existence of domains between actively transcribing genes. The 3D (as opposed to genomic) distance between the TSSs of two actively transcribing genes or between an actively transcribing gene and some sort of obstruction is captured by the DNA mechanical framework utilized in this letter. In the absence
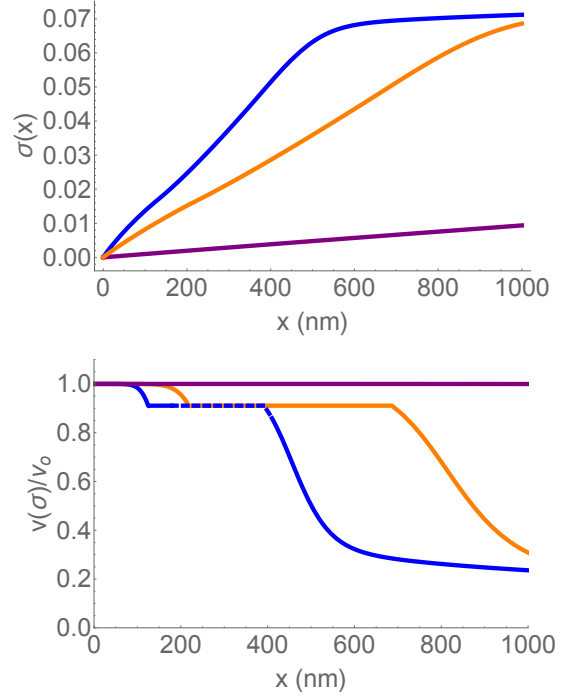

FIG. 4: (color online) Supercoiling σ and velocity $v(\sigma)$ as a function of RNA elongation x for $L = 5, 10, 100$ μm (blue, orange, purple) with $\alpha = \frac{1}{2}$, $\eta v = 1$ at a fixed force of $F = 1/2$ pNnm ($S = 582.0$, $\tau_0 = 9.5$, $P = 189.6$ pnNm) for a system with a torque dependent velocity. For each length the torque cutoff was set at $\tau_c = 12 pNnm$.

of adequate relaxation two actively transcribing adjacent genes with opposing orientation will generate increased SCD in the region of DNA between them. If in this region $|\sigma| > |\sigma_s^*|$ the TSSs, and the area between them, will have increased 3D proximity [14]. For a piece of DNA in the regime $|\sigma| > |\sigma_s^*|$ there will be very little 3D distance between the two TSSs. Therefore, if the value of the constant torque regime τ_o is below that of the operational limit of the RNAP τ_c and $x_g > x_p^*$ the 3D distance between the TSSs of two active genes (or obstacle) can be point like. This behavior is a possible explanation for the existence of DNA domains in bacteria [5] and eukaryotes [17] as well as a possible mechanism for the formation of transcription factories [15, 18].

Thus, it is important to construct a framework which can accommodate simultaneously active RNACs for both the same gene as well as for neighboring genes. It is straightforward to introduce additional torque constraints on a given piece of DNA allowing for the incorporation of the action of multiple RNAC on the same gene as well as multiple barriers to DNA rotation. A model for a supercoiling dependent multi-gene system without mechanical arrest or explicit physical components of transcription has already been proposed [19]. Using elements constructed here it is possible to imagine a system where the relative rotation of multiple RNACs is determined by
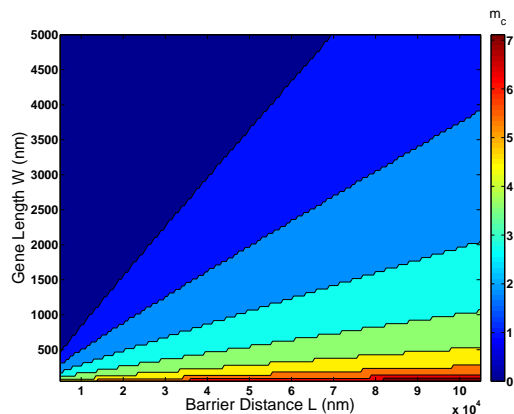
FIG. 5: (color online) Figure shows the number of transcripts m_c that can be made for a gene of length W against a barrier of length L with no relaxation between transcription events. The mechanical values used are $\alpha = \frac{1}{2}$, $\eta v = 1$ at a fixed force of $F = 1/2$ pNnm ($S = 582.0$, $\tau_0 = 9.5$, $P = 189.6$ pnNm) for a system with a torque dependent velocity.

the mechanical state of shared DNA. Each RNAC will operate using the basic coordinate system and torque balance outlined in equations 1 and 2. For a system of more than two genes dynamic simulations will likely be needed. A full description of a multi-gene system will benefit from measurements of the basic elements outlined in this letter and a detailed analysis of such a system is left for future work.

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