Assignment #1

Physics 498: Statistical Physics of Biological Complexity and Information

Micromechanics of RNA Polymerase

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Rahul Roy
Center for Biophysics and Computational Biology
University of Illinois at Urbana-Champaign
email: rroy1@students.uiuc.edu
Introduction
RNA Polymerase (RNAP) plays a significant role in the sustenance and propagation of life by acting as the transducer of the genetic information to its expression in all biological forms. At the molecular level, RNAP results in the synthesis of an RNA transcript of the template DNA [Erie DA et al., 1992]. The molecular mechanism by which the RNAP moves along the DNA template, synthesizing a RNA chain by converting the free energy of the nucleotide binding and hydrolysis into a force directed along the DNA axis has been an interesting and hot topic both experimental and theoretical research [Wang H et al., 1998; Wang MD et al., 1998]. In this work, the energetics based model developed by George Oster and co-workers is reviewed [Wang H et al., 1998] and an experimental set up to test the reported step size $\delta$ [Yin H et al. 1995] of the RNAP motion along the DNA, is proposed.

Method
First I will explain the proposed experimental set up and then discuss the RNAP micromechanics model under review in its context. In the proposed experiment, the DNA micromanipulation technique using magnetic traps [Strick T, 1999] has been modified to study the force generation in RNA Polymerase and its step size during transcription. The RNAP is affixed to the substratum, and a magnetic bead attached to the end of the DNA is held in a magnetic field. When nucleotide is added to the solution, the RNAP exerts tension on the DNA strand that pulls the bead. The location of the bead can be tracked with $10^{-8}$ m precision using the CCD camera set up, the force exerted on the bead by the RNAP under equilibrium can be accurately measured.

Model
Now I shall discuss the geometry of the model (Fig.1). The DNA strand is attached at one end to the bead, which is held in the magnetic trap. The other end within the RNAP is annealed to the growing RNA strand via the 8-12-bp hybrid in the transcription bubble. The RNA strand, in turn, can make contact with the RNAP at a site at the front of the catalytic site (labeled F in Fig. 1). This site acts as a "barrier" against which the tip of the transcript can fluctuate. In the model, F is the site where tension in the DNA strand is transferred to the RNAP, while allowing the template DNA strand to pass freely. Here the "barrier" F represents the overall interaction between the RNAP and the DNA-RNA hybrid, which prevents the DNA-RNA hybrid from being pulled out of the RNAP by the load force. In the transcription process, RNAP needs to work against the load force and overcome the attachments of RNAP to DNA and RNA. It does this by rectifying the Brownian diffusion against the "barrier" F using the free energy of nucleotide triphosphate (NTP) binding and hydrolysis. Thus a dominant portion of the load force should flow through the "barrier" F to the substratum and then back to the magnetic trap. The RNAP is modeled as an elastic body and the DNA, RNA and the magnetic trap are assumed to be springs.
Assumptions and Justification
Without delving deep in the mathematical derivations, I would point out the salient assumptions of the model.

1) The motion of the RNAP along the DNA is modeled as a discrete state Markov chain. Figure 2 shows the spatial displacement, \( n \), of the RNAP along the horizontal axis and the reaction coordinate along the vertical axis. All transition rates with a horizontal (\( n \)) component depend on the load force, \( f \), from the magnetic trap. The origin of the coordinate system is placed at a particular nucleotide (e.g., the first) added to the growing RNA chain, and denoted by \( n \) the position of the RNA transcript tip. The subscript refers to the length of the transcript (or equivalently, the position of the RNAP from the beginning of the transcript). The assumption can be justified because the rate of relaxing to the thermodynamic equilibrium after the addition of the nucleotide is much faster than both the nucleotide insertion rate and the pyrophosphate release rate. In this simplified model of the polymerization kinetics, an RNAP containing a transcript of length \( n \), can exist in two polymerization states:

- \( P_n \) (containing a transcript of length \( n \) with no PP\(_i\) bound)
- \( P'_n \) (containing a transcript of length \( n \) with PP\(_i\) bound).

2) The model developed treats the DNA as a homo-polymer with only one nucleotide type. The incorporation of sequence dependence in the model will require the relative strengths of the bonds holding the terminal nucleotide onto the end of the transcript.

Model equations
1) Markov equation: The governing equations for the Markov chain in Figure 2. can be where \( \sum \rho_1(n, t) + \sum \rho_2(n, t) = 1 \). The solution to these equations will provide the force-velocity curve

\[
\frac{d\rho_1(n, t)}{dt} = \rho_1(n + 1, t)\beta_4 + \rho_2(n + 1, t)\beta_1 + \rho_2(n, t)\alpha_2 \\
+ \rho_1(n - 1, t)\alpha_3 - \rho_1(n, t)(\alpha_4 + \alpha_1 + \beta_2 + \beta_4)
\]

\[
\frac{d\rho_2(n, t)}{dt} = \rho_2(n + 1, t)\beta_3 + \rho_1(n, t)\beta_2 + \rho_1(n - 1, t)\alpha_4 \\
+ \rho_2(n - 1, t)\alpha_3 - \rho_2(n, t)(\alpha_3 + \alpha_2 + \beta_1 + \beta_2)
\]

2) Corresponding to the model in Figure 2 the following expression for the load-velocity curve is obtained, where alpha and beta represent the rate parameters:

\[
v = \frac{\alpha_1\alpha_2 - \beta_1\beta_2 + (\alpha_1 + \beta_2)(\alpha_3 - \beta_3)}{\alpha_1 + \beta_1 + \alpha_2 + \beta_2}
\]
3) Independent information about the motor function is contained in the statistical variance of the motor’s motion. This variance can be characterized by an "effective" diffusion constant, $D_{\text{eff}}$, given by

$$D_{\text{eff}} = \delta^2 \left[ \frac{\alpha_1 \alpha_2 + \beta_1 \beta_2 + (\alpha_1 + \beta_1)(\alpha_3 + \beta_3) + (\beta_1 + \alpha_2)}{(\alpha_4 + \beta_1) - 2(\alpha_3 - \beta_3)(\alpha_4 - \beta_4)} \right]$$

$$- (\alpha_1 \alpha_2 - \beta_1 \beta_2 - (\alpha_1 + \beta_2)(\alpha_4 - \beta_4) - (\beta_1 + \alpha_2)(\alpha_3 - \beta_3))$$

$$\times \frac{\alpha_1 \alpha_2 - \beta_1 \beta_2 + (\alpha_1 + \beta_2)(\alpha_3 - \beta_3)}{(\alpha_1 + \beta_1 + \alpha_2 + \beta_2)^2}$$

The $D_{\text{eff}}$ is related to the variance by the following relation:

$$\text{var}(x(t)) = \langle x(t)^2 \rangle - \langle x(t) \rangle^2 = 2 D_{\text{eff}}$$

This relation is used to calculate the $D_{\text{eff}}$ from the variance and then the step size $\delta$ is calculated.

**Results**

1) The force vs velocity curves for RNAP were found to be concave in nature (also found in laser trap experiments), i.e. the velocity remains constant up to loads above 20pN and after that falls off to a stall load between 25 to 30pN. This is highly uncharacteristic of molecular motors which generally show a convex or linear behavior. The proposed model in the paper seems to explain the phenomenon on the basis of the slow (relative to the mechanical motions) rate of pyrophosphate release, which is the rate-limiting step in transcription. So at low forces velocity is determined by the PP$_i$ release rate (which is load independent) but as the load rises enough to increase the time scale of the mechanical relaxation to the order of the PP$_i$ release, the rate of the process drops to zero.

2) The model also predicted the effect of concentrations of nucleotides and pyrophosphates in the solution. When the concentration of PP$_i$ is increased from 1 µM to 1 mM, the stall force is virtually unchanged, whereas the maximum velocity is reduced by half. When the concentration of NTP decreases, the stall force decreases by roughly the same percentage as the maximum velocity.

**Discussion**

The mechano-chemical model for RNAP developed by Oster and co-workers has been able to explain the concave nature of the force-velocity curves on the basis of the relatively small time scale of the mechanical relaxation of the RNA tip at the RNAP catalytic site. The model also accounts the changes in the stall force and effective diffusion with changes in the nucleotide and pyrophosphate concentration in close
agreement to experimental results. The model presented here provides an explanation for the energy transduction process of the RNAP propagation during transcription; it is essentially an extension of the Brownian ratchet polymerization models developed earlier [Mogilner A, and Oster G, 1996; Peskin CS et al., 1993]. The model demonstrates that a Brownian ratchet mechanism with a step size of one nucleotide is sufficient to account for the sizable stall force measured in the laser trap experiments.

In experimental magnetic trap set up, variance measurements will be used to determine the $D_{\text{eff}}$ and which is used further to calculate the step size of the RNAP. A similar parameter, randomness parameter $r$, has been used earlier to determine the step size of the kinesin protein [Schnitzer MJ and Block SM, 1995].

**Bibliography**