A chemical kinetic model of the elongation dynamics of RNA polymerase along a DNA sequence is introduced. The proposed model governs the discrete movement of the RNA polymerase along a DNA template, with no consideration given to elastic effects. The model’s novel concept is a “look-ahead” feature, in which nucleotides bind reversibly to the DNA prior to being incorporated covalently into the nascent RNA chain. Results are presented for specific DNA sequences that have been used in single-molecule experiments of RNA polymerase along DNA. By replicating the data analysis algorithm from the experimental procedure, the model produces velocity histograms, enabling direct comparison with these published experimental results.

PACS numbers:
RNA polymerase is the key enzyme of transcription, the step at which most regulation of gene expression occurs. Transcription consists of three distinct processes: initiation, elongation and termination. Of these processes, elongation has been the least studied because conventional experimental biological techniques have been unable to investigate the dynamical properties of RNA polymerase during transcriptional elongation. Fortunately this situation has changed with the advent and extensive use of single molecule force microscopy.

From a modeling perspective, elongation is the process in transcription most amenable to a quantitative description. RNA polymerase elongation dynamics can be seen as a stochastic process, more specifically as a one-dimensional random walk of the polymerase molecule along the DNA. A stochastic model for the motion of RNA polymerase was proposed by Julicher and Bruinsma. Others, most notably Wang et al, have studied force generation of RNA polymerase during elongation by approximating the internal strain of RNA polymerase using the concept of springs.

In this letter, then, we introduce a formal chemical kinetic model for the dynamics of the movement of RNA polymerase along DNA. In our model we make no reference to continuous motions and focus instead on the discrete events of reversible binding and unbinding of nucleotides to the DNA, and on the covalent linkage of nucleotides into the nascent RNA chain. We apply this model to specific DNA sequences that have been used in actual experiments.

During elongation, the double stranded DNA is locally melted by the RNA polymerase over a distance of approximately 14 - 17 basepairs. This locally melted region is known as the transcription bubble. Within the transcription bubble, one strand of the DNA acts as a template, upon which complementary ribonucleotide triphosphates (ATP, GTP, CTP, and UTP) can reversibly bind and unbind to/from the DNA template strand. It has been hypothesized, however, that only a part of the transcription bubble is actually used for transcription. The size of this window of activity within the transcription bubble formed by the RNA polymerase is an integer parameter of our model. The binding of ribonucleotides within the window of activity is assumed to be reversible.

An irreversible reaction, however, is the incorporation of a ribonucleotide into the nascent RNA chain. This can occur only when that ribonucleotide is reversibly bound at the first site of the window of activity, i.e., the site at the 3' end of the nascent RNA chain. When such in-
FIG. 1: (a) The figure shows the look-ahead window of RNA polymerase. Since the first site (left end of box, indicated by tic mark) is unoccupied the polymerase cannot move forward. Possible events are the unbinding of C, G, or U, or the binding of any ribonucleosidetriphosphate to any of the 5 unoccupied sites. (b) Same as (a) except that the first site within the lookahead window is also occupied. Possible events include, as in (a), the unbinding of any of the reversibly bound ribonucleotidetriphosphates or the binding of any ribonucleotidetriphosphates (including incorrect Watson-Crick basepairing) to any of the unoccupied sites. In this case, however, there is an additional possible event because the first site is occupied, namely, the forward motion of RNA polymerase, as depicted by the arrow in the figure. Note, in particular, that after this motion the new first site in the window may again be occupied (as shown) leading to the possibility of another forward step as a subsequent event.

Corporation of a ribonucleotide into the nascent RNA chain occurs, we assume that the RNA polymerase (and hence the transcription bubble and the window of activity) translocates forward one basepair. Because the window of activity has a size of more than one basepair, it is quite likely that when the polymerase molecule, and hence the window, moves forward, it will already find the correct nucleotide bound at what has just become the site where that nucleotide can be incorporated into the growing RNA chain. This is the 'lookahead' feature of the model, a kind of parallel processing: placement of the correct ribonucleotidetriphosphate at each site on the template strand of the DNA can occur before that site has been reached by the nascent RNA molecule.

The model is completely specified, then, by the following parameters: $w =$ length (in basepairs) of the lookahead window, $(K_{on})_{ij} =$ rate constant for reversible binding of ribonucleotide of type $i$ (ATP, CTP, GTP, or UTP) to deoxyribonucleotide of type $j$ (A, C,
G, T) in the template strand within the window of activity; \((K_{\text{off}})_{ij}\) = rate constant for unbinding of reversibly bound ribonucleotide of type i from deoxyribonucleotide of type j; \((K_{\text{forward}})_{ij}\) = rate constant for covalent incorporation of ribonucleotide of type i into the nascent RNA chain, provided that there is a ribonucleotide of type i reversibly bound to a deoxyribonucleotide of type j at the first site or the window of activity. Note that we consider not only correct Watson-Crick basepairings, but also the possibility of errors. The parameter \((K_{\text{on}})_{ij}\) is of course, much larger, and \((K_{\text{off}})_{ij}\) much smaller when, \((i,j)\) is a correct Watson-Crick basepair than otherwise. This mechanism protects against errors in transcription. Further error protection could be obtained by making \((K_{\text{forward}})_{ij}\) larger when \((i,j)\) is a correct Watson-Crick basepair then when it is not. In our simulations, however, we have assumed that \(K_{\text{forward}}\) is constant, independent of \((i,j)\).

We model the movement of RNA polymerase along DNA using the Gillespie algorithm. For every possible transition a suitable rate constant is assigned: for each unoccupied site within the window of activity, there are 4 binding rate constants, one for each of the ribonucleotidetriphosphates that can possibly occupy that site; if a site is occupied within the window of activity, then there is a rate constant for the ribonucleotidetriphosphates on that site to come off; if the first site within the lookahead window is occupied, then there is a rate constant for the RNA polymerase to translocate forward one basepair.

The Gillespie algorithm jumps from event to event. Let \(t^n\) be the time of the nth event. Immediately after the nth event, let the system be in a state such that \(m^n\) different transitions are possible (where the superscript n is just a label, not a power), and let the rate constants for those transitions be \(k_1^n \ldots k_m^n\). Each of the k’s is selected from one of the \((K_{\text{forward}})_{ij}\), \((K_{\text{on}})_{ij}\), or \((K_{\text{off}})_{ij}\) as appropriate. Note that \(m = 4u + (w-u) + b\), where w is the window size, u is the number of unoccupied sites, and b = 1 if the first site is occupied and b=0 otherwise. Then random time intervals \(T_1^n \ldots T_m^n\) are chosen according to

\[
T_j^n = \frac{-\log(R_j^n)}{k_j^n} \quad j = 1 \ldots m
\]  

where the \(R_j^n\) are independent random numbers uniformly distributed on (0,1]. Then the time of the next event is chosen as
Table I: Values of the rates \((K_{\text{forward}}}_{ij})\), \((K_{\text{on}}}_{ij})\), and \((K_{\text{off}}}_{ij})\) and window size used in the ‘look-ahead’ simulations. i and j refer to ribonucleotidetriphosphate (ATP, CTP, GTP or UTP) and deoxyribonucleotide (A, C, G, or T) respectively. These parameter values were chosen arbitrarily to explore the model’s behavior.

\[
t^{n+1} = t^n + T^n, \quad \text{where} \quad T^n = \min_j T_j^n
\]

and the index \(J^n\) of the transition that occurs is chosen as the value \(j\) that achieves the minimum.

We applied our model to a specific DNA sequence that was used in actual experiments [2]. For elongation velocity analysis, we imitated the algorithms used in the experimental procedure found in [2] to analyze our data and thereby obtained velocity profiles as well as histograms for the distribution of the velocities. Results of our elongation simulations are shown in Fig. 2 while the analysis of transcriptional velocity is shown in Fig. 3.

Because our model involves chemical kinetics only, and does not commit to any detailed physical mechanism, it is consistent either with powerstroke models such as [20] and [11] or Brownian ratchet models such as [8]. One definite assumption, however is that the polymerase motion is unidirectional. We argue that backwards translocation is uncommon for several reasons: (1) the breaking of a covalent bond of the nascent RNA chain is energeti-
FIG. 2: Results of simulation along the DNA tether used in [2]. (a) Here we have 10 elongation profiles of RNA polymerase traversing along the DNA tether. Experimentally, the RNA polymerase attaches randomly to a location on the DNA strand and then begins the process of transcription; our model reflects this fact. (b) Closeup of one of the profiles in (a). Note the pause duration of about 1 to 2 seconds, followed by an activity of rapid translocation. This pause could be indicative that the RNA polymerase is waiting for the first site in the window of activity to be occupied. In our model, it is possible that certain DNA sequences are more amenable to pausing than other sequences.

Eventually unfavorable; (2) at certain sites, the folding of the nascent RNA chain into a hairpin provides a 'backstop' that prevents the nascent RNA chain from moving backwards (3) backwards translocation occurs only under special circumstances, namely during transcriptional arrest/termination or a complete absence of NTPs [13] [14].

The nature of pauses in the motion of RNA polymerase has been much debated. Pausing is important to understand because it can enables synchronization to the enzymatic events of translation and regulates the overall speed of transcription. Recent single molecule experiments on transcriptional elongation [13] [2] [9] [7] have all reached different results and conclusions concerning the nature of pausing. Forde et al [9] has hypothesized that elongation is a bipartite mechanism, in which the RNA polymerase backtracks followed by a conformational change of the polymerase complex, which results in an arrested molecule incapable of being rescued by an assisted mechanical force. Bai et al [3] and Shundrovsky et al [17] have hypothesized that pausing is the result of backwards translocations along the DNA. Neuman et al [13] and Shaevitz et al [15] have hypothesized that a structural rearrangement within the RNA polymerase enzyme is the cause of short pausing. Based on
FIG. 3: (a) Simulation of an elongation profile of a typical RNA polymerase along a specific DNA tether used in [2]. (b) Linear least squares Gaussian fit of the elongation profile in (a) using the algorithm found in [2] to obtain a velocity profile. (c) Normalized distribution of the velocity for single RNA polymerase elongation profile of (b). (d) Combined normalized distribution for 30 RNA polymerase runs along the DNA tether. The interested reader should compare this figure with figure 2 ('Analysis of Elongation Velocity') found in [2]. Note: all simulations are with an 8 basepair window of activity.

In our model, the statistics of the motion of RNA polymerase may be described as follows. Consider the limit in which the forward rate constant is very fast. Then RNA polymerase moves forward every time that the first site within the lookahead window becomes occupied. The distribution of the waiting time for this to occur will be exponential with a rate constant that may be sequence-dependent. Once a forward step does occur, it may be immediately followed by one or several additional forward steps, depending on how many adjacent sites

the latter experiments, the majority of pausing has been shown to be short and ubiquitous, and is not the result of backtracking along the DNA; on the other hand, longer pauses are hypothesized to occur by an entirely different mechanism.
within the lookahead window happened to be filled at the moment when the first site is filled. Put another way, the RNA polymerase 'slides' the length of the adjacently occupied nucleotides. Such sliding is consistent with the inchworm model \[6\]. An interesting property of the lookahead model that we have not yet fully explored is the potential role of the lookahead feature in preventing transcription errors. Assuming that there is a nonzero probability of incorporating an incorrect ribonucleotide covalently into the nascent RNA chain, it becomes important to reduce the probability of such an incorrect base being present at the site where it would be incorporated. This may be accomplished by having a high off-rate for incorrect basepairings, and by allowing sufficient time for this off rate to be effective. The lookahead model provides this possibility (in contrast to a model which only involves binding followed by a covalent linkage).

We have presented a chemical kinetic model for RNA polymerase translocation using the same DNA sequence that was utilized in actual experiments. The model can be seen as formal since we focus on discrete kinetic events, while ignoring more 'continuous' effects such as elasticity. The assumption of forward translocation and the nature of pausing in our model are consistent with the results found in \[13\] and \[15\]; the work of \[11\] supports the biological basis of our model. Finally, and most importantly, the output of the model can be processed, by replicating the data analysis algorithms used in the experimental procedure, to produce velocity histograms allowing for direct comparison with experimental results.

Future work includes: 1) fitting of our simulation results to experimental data to obtain a set of best parameters, which can then be used to test the validity of the model with data from future experiments 2) characterizing temporal correlations and statistics of jump size 3) studying error-correcting mechanisms 4) incorporating nearest neighbor effects in the unbinding and binding of ribonucleotidetriphosphates. In conclusion, we are only at the beginning of exploring this model, and we hope to address these aforementioned issues in a future publication.

We thank the Center for Applied Mathematics at Cornell University for the generous use of computing resources during this project. We would also like to thank Arthur LaPorta and Lu Bai at LASSP/Cornell University for information concerning their single molecule experiments and Udo Wehmeier for preliminary work on the model of this paper. The primary author was supported on an NSF-IGERT grant (DGE-033366).
* Electronic address: yamada@cam.cornell.edu

[1] E.A.Abbondanzieri, W.J.Greenleaf, J.W.Shaevitz, R.Landick, and S.M.Block, *Nature* **438**, 460 (2005).

[2] K. Adelman, A. LaPorta, T Santangelo, J. Lis, J. Roberts, and M Wang *Proc. Natl. Acad. Sci. USA* **99**, 13538 (2002).

[3] L. Bai, A. Shundrovsky, and M. Wang. *J.Mol.Biol.* **344** 335 (2004).

[4] C. Bustamante, J. Macosko, and G. Wuite, *Nature Reviews* **1**, 130 (2000).

[5] C. Bustamante, Z. Bryant, and S. Smith *Nature* **421**, 423 (2003).

[6] M. J. Chamberlin, *The Harvey Lectures* **88**, 1 (1995).

[7] R. Davenport, G. Wuite, R. Landick, and C. Bustamante *Science* **287**, 2497 (2000).

[8] R. Ebright, *Lectures at ITP: Biomolecular networks 2003*. Lectures available on the web at [http://online.kitp.ucsb.edu/online/bionet03/ebright](http://online.kitp.ucsb.edu/online/bionet03/ebright).

[9] N. Forde, D. Izhaky, G. Woodcock, G. Wuite, and C. Bustamante *Proc. Natl. Acad. Sci. USA* **11682**, 99 (2002).

[10] D. T. Gillespie, *J. Computational Phys.* **4**, 403 (1976).

[11] X.Q. Gong, C. Zhang, M. Feig, and Z.F. Burton, *Molecular Cell* **18**, 461 (2005).

[12] F. Julicher and R. Bruinsma, *Biophysical Journal* **74**, 1169 (1998).

[13] K. Neuman, E. Abbondanzieri, R. Landick, J. Gelles, and S. Block *Cell* **437**, 115 (2003).

[14] E. Nudler, A Mustaev, E. Lukhtanov, and A. Goldfarb *Cell* **89**, 33 (1997).

[15] J.W. Shaevitz, E.A. Abbondanzieri, R. Landick, and S.M. Block, *Nature* **426**, 684 (2003).

[16] T. Strick, J. Allemand, V. Croquette, and D. Bensimon *Physics Today* **October**, 46 (2001).

[17] A. Shundrovsky, T.J. Santangelo, J.W. Roberts, M.D. Wang, *Biophys. Jour.* **87**, 3945 (2004).

[18] M. Wang, M. Schnitzer, H. Yin, R. Landick, J. Gelles, and S. Block *Science* **282**, 902 (1998).

[19] H. Wang, T. Elston, A. Mogilner, and G. Oster *Biophysical Journal* **74**, 1186 (1998).

[20] Y.W. Yin and T.A. Steitz, *Cell* **116**, 393 (2004).