Structurally specific thermal fluctuations identify functional sites for DNA transcription

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We report results showing that thermally-induced openings of double stranded DNA coincide with the location of functionally relevant sites for transcription. Investigating both viral and bacterial DNA gene promoter segments, we found that the most probable opening occurs at the transcription start site. Minor openings appear to be related to other regulatory sites. Our results suggest that coherent thermal fluctuations play an important role in the initiation of transcription. Essential elements of the dynamics, in addition to sequence specificity, are nonlinearity and entropy, provided by local base-pair constraints.

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One of the most challenging subjects in biophysics is the relation between biomolecular motions and function \[^{[1]}\]. We present an example suggesting that functionality arises from structurally coherent dynamics, with essential ingredients of: sequence-specificity, nonlinearity and entropy; the nonlinearity form local constraints is crucial. In particular, remarkably successful comparisons of numerical simulations of a minimal model (see below) of transverse dynamics for gene promoter DNA segments with in vitro transcriptional experiments, shows that the combination of all the above mentioned components controls coherent “bubble” fluctuational openings of base-pairs around specific sites of promoter DNA. Remarkably, the prominent opening occurs at the transcription initiation site, while minor openings coincide with regulatory sites at which transcription factors and other assisting proteins are bound. These results demonstrate the importance of the sequence structure, not simply as a static and passive element, but to provide the template for specific coherent fluctuations determining function. These coherent structures constitute a colored spatio-temporal stochastic environment. This is an example of the importance of a (dynamic) landscape of substates \[^{[1]}\].

We have used a microscopic model proposed by Peyrard and Bishop \[^{[2]}\] to describe the dynamics of the openings of double stranded DNA. This model focuses only on the most relevant degrees of freedom, namely the transverse stretching of the hydrogen bonds connecting complementary bases in the opposite strands of the double helix. Its reduced character, involving a small number of variables, makes it suitable for simulations over relatively long times and appropriate for gathering sufficient statistics. Subsequent key improvements \[^{[3]}\] succeeded in reproducing the abrupt (first order) character of the observed DNA denaturation transition. The potential energy of this model reads \[^{[3]}\].

\[
V = \sum_n \left[ D_n \left( e^{-a_n y_n - 1} \right)^2 + \frac{k}{2} \left( 1 + \rho \exp \left[ -\beta (y_n + y_{n-1}) \right] \right) (y_n - y_{n-1})^2 \right] (1)
\]

Here the sum is over all the base-pairs of the DNA and \( y_n \) denotes the displacement from the equilibrium position of the relative distance between the bases within the \( n \)th base-pair, divided over \( \sqrt{2} \). The Morse potential (other similar potentials can also be used) in the first term provides the effective interactions between complementary bases; it represents both the attraction due to the hydrogen bonds forming the base-pairs and the repulsion of the negatively charged phosphates in the backbone of the two strands screened by the surrounding solvent. Beyond the exact details of this interaction, an important issue is a correct description of the nonlinearities. The parameters \( D_n \) and \( a_n \) of the on-site potential distinguish between the two possible combinations of bases, i.e. adenine-thymine (A-T) or guanine-cytosine (G-C), at site \( n \), depending on the particular sequence. The second term in the total potential energy represents the stacking interaction potential between adjacent base-pairs. Here the nonlinear inter-site coupling, given by the exponential term that effectively modifies a harmonic spring constant, is essential for representing local constraints in nucleotide motions, which result in long-range cooperative effects \[^{[3]}\]. As in elastic materials \[^{[1, 3]}\], it controls lattice vibrations, yielding accurate entropic terms \[^{[3]}\]: the stiffening of the coupling in the compact state compared to that in the open state leads to an abrupt entropy-driven transition \[^{[3]}\]. Physically, the constraint describes the change of the next-neighbor stacking interaction due to the distortion of the hydrogen bonds connecting a base-pair, mediated through the redistribution of the electrons on
Despite its simplified character, this model has successfully reproduced not only the sharp melting (denaturation) transition occurring when the two strands of the DNA separate from each other as temperature increases, but also the details of the precursor (nucleation) fluctuational openings and the dynamics upon approaching the denaturation transition. The coexistence of two essential features are necessary for obtaining the first-order-like transition: the nonlinear coupling constant that decreases in the denaturated phase providing an increase in entropy, and a plateau in the on-site potential which should not increase unbounded. Regarding the precursor fluctuations, intrinsic localized modes nucleate as nonlinear bubble opening events that subsequently interact and grow, providing the experimentally observed denaturation bubbles. This nucleation regime, precursor to the melting, extends over temperatures several tens of Kelvin below the melting transition (the biologically relevant regime).

In addition to capturing the essential features of thermally induced denaturation of long DNA chains, the model has been used to reproduce the melting curves of very short heterogeneous DNA segments, in excellent quantitative agreement with experimental data. Furthermore, it provides the characteristic multi-step melting observed in single heterogeneous DNA molecules. Recently, the model has been used to investigate charge transport properties in a flexible DNA chain, where the charge is coupled to the lattice degrees of freedom. The bubbles, as relatively long-lived intrinsic inhomogeneities (“hot-spots”), represent a colored noise environment, which qualitatively influences charge dynamics.

Motivated by the successful description of the nonlinear thermal fluctuations, we have applied this model to explore the possible role of the intrinsic bubble openings for the transcriptional initiation and regulatory sites of specific promoter DNA sequences. In particular, we have studied the adenoassociated viral P5 promoter (P5), the adenovirus major late promoter (AdMLP) and the bacteriophage T7 core promoter (T7). The base-pair sequences of these promoters are presented in Fig. 1. \textit{In vitro} transcription experiments demonstrating the specific initiation of RNA polymerase II transcription from DNA templates containing the corresponding promoter fragments are shown in Fig. 2. For the experimental details see reference.

We performed Langevin molecular dynamics (thereby capturing thermal fluctuation and dissipation effects) for nucleotides of mass $m$ evolving in the potential $V$ of equation (1). We have used the parameter values given in reference: $k = 0.025 \text{ eV}/\text{A}^2$, $\rho = 2$, $\beta = 0.35 \text{ A}^{-1}$ for the inter-site coupling, while for the Morse potential $D_{GC} = 0.075 \text{ eV}$, $a_{GC} = 6.9 \text{ A}^{-1}$ for a GC base-pair, and $D_{AT} = 0.05 \text{ eV}$, $a_{AT} = 4.2 \text{ A}^{-1}$ for an AT pair. The simulated temperature was 300 K (below the melting temperature but in the precursor regime of bubble formation).

The statistics of the thermally induced openings was obtained using 100 different realizations for each DNA sequence studied. We ran each realization for 1 ns, after reaching thermal equilibrium, and monitored the state of the system every 1 fs. Thus we have $10^6$ events for each one of the 100 realizations. At every event we checked the corresponding bases.

FIG. 1: Base-pair sequences of the studied DNA gene promoter fragments. \textbf{a)} 69 base-pair long viral P5 promoter, \textbf{b)} 86 base-pair long viral AdMLP promoter, and \textbf{c)} 70 base-pair long bacterial T7 promoter.
FIG. 2: Auto-radiography of $[^{32}P]$-labeled reverse transcripts after separation by gel electrophoresis (lanes 3). Arrows indicate the direction of specific transcription started from the initiation site +1 in all cases. Lanes 1 indicate base position markers obtained by chemical sequencing. (a) P5 promoter, (b) AdMLP promoter, and (c) T7 promoter. Lane 4 in (a) shows elimination of the transcription for the mutated P5 promoter, where the nucleotides at +2 and +3 have been changed from AT to GC.

FIG. 3: Logarithm of the probability $P$ for the occurrence of an opening of 10 base-pair width and amplitude of more than 2.1 $\AA$ (thick solid line) or 1.4 $\AA$ (dotted line) starting at a particular site $n$ of the DNA fragment, as a function of $n$, for the (a) P5 promoter, (b) AdMLP promoter, and (c) T7 promoter.

displacements $y_n$ of the base-pairs at each site $n$ and the following $m - 1$ ($m$ varying from 1 to 19) base-pairs. If the openings at all these $m$ subsequent sites are greater than a threshold value $y_{th}$ (varying from one tenth to few $\AA$) we assign a contribution to the opening event at the $n$th base-pair of the sequence. The obtained opening probabilities along the studied DNA segments for bubble sizes of $m = 10$ base-pairs and thresholds $y_{th} = 1.0$ and 1.5 $\AA$ for accepting an opening are presented in Fig. 3. (We recall that the real openings are equal to $y\sqrt{2}$). In all these cases the most probable openings are located at the transcription start site +1 (see Fig. 2). Furthermore, in the viral cases the other distinct openings seem to be related to known regulatory sites: in P5 the opening at the A/T rich region between -40 and -35 corresponds to the binding site of the transcription factor Yin Yang 1 [17], while in AdMLP the second higher opening is close to the binding site of the TATA-box binding protein [18], that is necessary for transcription. As can be seen from Fig. 3, openings of such large widths and amplitudes are rare events in our microscopic simulations, therefore requiring sufficient statistics.

We stress that similar local sequences do not exhibit the same opening probabilities; equal size segments of relatively weakly bound A/T pairs in different parts of the promoter show very different statistics (compare for example the region around -30 with that around +1 in P5). Furthermore, the larger openings do not occur in regions with longer A/T stretches, as might be intuitively anticipated because of the weaker bonding. Effective long range cooperativity (from the nonlinear inter-site potential in equation [11]) and competing localization lengths due to the spatial disorder and the nonlinearity are responsible for this high specificity: in general, length scale competition in nonlinear systems is known [17] to lead to complex spatio-temporal (dynamic landscape) behavior. The sensitivity of the cooperative/competing phe-
nomena, affected even by a single base-pair modification, enhances the predictive power of our model; a small mutation of the sequence (at a specific location) is sufficient to completely eliminate bubble formation at the transcription initiation site. In Fig. 4 we show numerical calculations of the opening probabilities for a mutated P5 promoter, where nucleotides +2 and +3 have been changed from AT to GC. This mutation completely eliminates the opening at the previous transcription start site, in agreement with no transcriptional events occurring in the corresponding experiment (see Fig. 2a, lane 4).

We emphasize that, as in previous applications of this model, the nonlinear inter-site coupling is crucial for its success \[3, 10\]. For example, as can be seen in Fig. 5, linearizing the stacking interaction term \( \rho = 0 \) results in very modified statistics for the openings of the P5 promoter, changes the position of the peaks along the sequence, and eliminates the successful comparison with the experimental observations. The nonlinear inter-site coupling constitutes a minimal representation of the local stacking constraint between neighboring base-pairs. As in more general situations of displacive structural phase transitions\[4, 5\], such local constraints can lead to long-range “elastic” interactions and macroscopic cooperativity.

In summary, our model and simulations suggest that structurally specific coherent thermal fluctuations identify locations in the DNA sequences where the RNA polymerase initiates transcription. Further, we find indications that the thermal fluctuations also help in recruiting other protein complexes participating in the transcriptional process, by separating the DNA double strand at specific locations. These bubbles precede protein binding and their possible role is limited to the very initial steps of the transcription. This suggests that DNA, through structurally specific dynamics, participates in directing its own transcription.

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