Salerno's Model of DNA Re-Analysed: Could Breather Solitons have Biological Significance?

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Abstract. We investigate the sequence-dependent behaviour of localised excitations in a toy, nonlinear model of DNA base-pair opening originally proposed by Salerno. Specifically we ask whether “breather” solitons could play a role in the facilitated location of promoters by RNA polymerase (RNAP). In an effective potential formalism, we find excellent correlation between potential minima and *Escherichia coli* promoter recognition sites in the T7 bacteriophage genome. Evidence for a similar relationship between phage promoters and downstream coding regions is found and alternative reasons for links between AT richness and transcriptionally-significant sites are discussed. Consideration of the soliton energy of translocation provides a novel dynamical picture of sliding: steep potential gradients correspond to deterministic motion, while “flat” regions, corresponding to homogeneous AT or GC content, are governed by random, thermal motion. Finally we demonstrate an interesting equivalence between planar, breather solitons and the helical motion of a sliding protein “particle” about a bent DNA axis.

Key words: DNA soliton, RNA polymerase, sliding, bacteriophage T7

1. Introduction

Protein-DNA interactions play many of the fundamental roles in gene regulation. An understanding of the mechanisms involved in these processes is one of the major current goals for numerous biological sciences. With large repositories of genetic information available – and costs associated with difficult, highly specific experiments – the question of how well such molecular interactions can be simulated is clearly important to investigate. Enzymes and many transcriptional factors are proteins, often composed of tens to hundreds of amino acids, while the DNA domains to which they bind can contain, in the case of prokaryotes, up to 10^5 nucleotide bases. All-atom modelling of such a molecular complex, even neglecting the key roles of hydration and ions, is beyond current computational ability.

An alternative, logical first step is to consider one specific kind of interaction, focussing exclusively on its salient features, and develop an accordingly simplified model. In this spirit, simple dynamical models of DNA have been studied for almost two decades, most successfully in regard to describing denaturation

experiments (see Ref. [1] for a review). The process by which the motions of small DNA molecules containing $\sim 10^6$ atoms are “coarsened” to the nucleotide base-pair level has previously been qualitatively argued [2]. Typically the DNA molecule is modelled by one degree of freedom per base-pair: a radial “stretching” [3] or a pendulum-like base “flipping” (Ref. [4] being a recent review).

We note, in this context, that *ab initio* calculations of small DNA oligomers [5] suggest that base-pair motions can be accurately approximated at the dinucleotide level in terms of two or three quasi-rigid, internal degrees of freedom, lending some weight to the coarse-graining assumptions previously made. The motivation for these simple models is that, if experimental results can be described with a small number of degrees of freedom, then these degrees of freedom must be the dominant ones for the process in question. The applications of such a model are necessarily restricted to extremely specific instances of DNA behaviour [2]. Given that many regulatory processes are governed by highly-specific, localised denaturation of the DNA helix, it is logical to investigate sequence-dependent dynamical behaviours in such a setting.

In 1991, Salerno [6] proposed a base-flipping model of the bacterial promoter DNA sequence A_1 in the T7 genome, suggesting the sequence had special, “dynamically active” qualities with regard to propagating kink solitons. Subsequent investigations of other host-specific promoter T7 sequences [7–9] resulted in similar findings. Moreover in Ref. [9] it was suggested that solitons could be created as conformational changes to the DNA helix due to DNA-RNAP (RNA polymerase) interactions. Recently the propagation of kinks through the entire T7 genome sequence has been studied [10], although the (significant) differences between host- and phage-specific promoter sequences were neglected. Another paper [11] investigated, whether kinks might propagate differently in coding and non-coding sequences.

Solitons had been previously suggested to have a role [12] in DNA transcription in the 1980’s; however, at least one picture which developed [13], of an RNA polymerase (RNAP) molecule “surfing” a thermally-driven region of open base pairs, is inconsistent with the known conformational changes of RNAP and DNA which occur during open complex formation. Secondly, no absorption resonances have been observed in microwave spectroscopy of DNA [14, 15]. Crucially the original motivation for invoking solitons, anomalously long lifetimes of DNA base-pair, openings, was shown to result from misinterpretation of data [16]. DNA solitons were vigorously dispelled by some researchers [17] with the result that they remain little more than a curiosity outside of the nonlinear physics community.

More generally, a variety of studies [18–20] have suggested connections between the base-sequence dependence of helical thermal stability and transcriptional regulation sites. The common feature in all these studies, soliton models included, is that AT-rich regions are distinguished by way of reduced thermal stability relative to GC-rich regions. Of course regions rich in AT might stand out from a regulatory

perspective for geometric, mechanical or chemical reasons. For example, tracts of A or T nucleotides carry intrinsic curvature [21] and confer flexibility to the DNA superstructure [22, 23], leading to significant departures from the average B-DNA form. Finally the role of counterions in determining DNA structure such as bending and groove width [24] and affinity for such tracts is not yet fully understood. Sequence-dependent variations in the electrostatic surface of DNA may also present a unique “signature” in promoter regions [25].

It should be emphasised that the solitons we consider have nothing to do with thermally-driven, transient base-pair openings, the source of the original controversy. Protein-DNA interactions involve conformational changes of the DNA helix and in our opinion it is logical to investigate, whether a small change might be modelled by a structural perturbation to a regular B-DNA helix, whether its translocation might be approximated by a soliton propagating through a nonlinear medium.

The structure of the paper is as follows: We briefly discuss aspects of protein-sliding and review the mechanism of lytic infection of *Escherichia coli* by T7 bacteriophage. Assuming DNA molecules can actually support nonlinear, quasi-solitonic excitations like those of the simple “base-flipping” model [6], we discuss the kind of biological roles they might serve. To this end we introduce the inhomogeneous Frenkel-Kontorova (IFK) model [26], the basis of Salerno’s approach [6–8], and its breather soliton solutions. The propagation of breather solutions is analysed via an effective potential formalism and we compute the energy landscape, comparing extrema with bacterial and phage promoters of the T7 genome. Supposing then, that solitonic excitations of DNA do not exist, we discuss alternative reasons why the correlations obtained between regulatory features and potential extrema might have been obtained. In particular a novel equivalence between base-flipping stability in the planar IFK model and bending in a 3-dimensional, helical model is outlined.

1.1. PROTEIN SLIDING

The mechanisms by which regulatory proteins, such as RNAP, can recognise their specific binding sites among tens, or even hundreds of thousands, of structurally identical nonspecific sites on a DNA strand are generally not well understood. A widely accepted hypothesis is that many proteins have several modes [27] with which to bind to DNA:

Nonspecific binding occurs when a polar domain of the protein displaces cations in the major/minor grooves of the DNA helix. The protein effectively slides, one-dimensionally, along the groove through a series of nonspecific binding events. The translocation mechanism for the nonspecific complex is not known, but is ATP-independent [25] and widely assumed to be driven by thermal motion. Other possible diffusion modes include “hopping”, where a nonspecifically bound protein dissociates and re-associates within the same DNA domain. For some proteins,

such as *lac* repressor [28], a possibility for transfer between sequentially-distant regions of DNA brought close together in 3-dimensional space also exists. In general, the facilitated location of an operator by a protein is likely to consist of a sequence of sliding, hopping or intersegmental transfer events [27]. A growing body of evidence exists that many regulatory proteins, such as repressors [28, 29], RNAPs [30–34], nucleases [35] and methylases [36] locate their operator sites in this way.

While the sliding component of facilitated target location is commonly assumed to be thermally driven, we point out that there is no experimental evidence to preclude dynamical effects resulting from local, sequence-dependent mechanical properties of DNA. In the seminal paper of Berg and co-workers [37] there are two assumptions, in particular, which may be unsatisfactory. Firstly the facilitated transport model is derived under the assumption of a homogeneous, free protein distribution. While such conditions can be arranged *in vitro*, this is not the case for a biological system. Secondly there is no real account of degrees of molecular recognition: operator sites are treated as “sinks”, defining a boundary condition for the one-dimensional diffusion equation. It is highly probable that some kind of “reading” process also occurs, mediated by the electrostatic interactions between protein residues and nucleotide functional groups lying in one, or both, of the grooves.

The limited empirical studies of sliding RNAP performed to date, for example Refs. [30–34], invariably average behaviour over many individual sliding events, masking any sequence-dependent variation which might exist. On the other hand, a recent model of the hypothetical reading process [38] was built upon the assertion that

...the protein should follow a noise-influenced, sequence-dependent motion that includes the possibility of slowing down, pauses and stops. . .

Let us qualitatively envisage, then, how soliton-like deformations might arise in RNAP-DNA interactions. The presence of enzymes as “mass defects” in a 1-dimensional DNA model have previously been considered [39–41] with regard to thermal breathers and transcribing RNAP. In distinction, initial binding to a non-specific DNA site entails insertion of polymerase domains into the major groove of the helix, where the displacement of counterions occurs. Suppose that the initial contact and recoil of RNAP during association induces a localised deformation in the B-DNA helix, which we approximate by a breather soliton. Breather excitations in the IFK model [26] are, due to the discreteness of the model, inherently unstable. Therefore the initially stationary breather would propagate along the strand, preferentially in a direction determined by local inhomogeneities in the base sequence. Further, the deformation is not a true soliton, owing to the discreteness of the DNA lattice, and radiates energy, eventually dissipating. In this regard we also note the mean sliding distance of RNAPs are known to be highly sensitive to variations in cation concentration [31, 32].

There are two pictures which are plausibly consistent with noisy, deterministic dynamics: either the RNAP can effectively “surf” the breather or that randomly moving RNAP and deterministically travelling breathers can interact somehow on collision. Regarding the latter, little is known about the structure of nonspecific protein-DNA complexes and for the remainder of the paper we consider the former, less speculative, scenario.

1.2. T7 BACTERIOPHAGE

Bacteriophage T7 is a member of the *Podovirales* family of viruses, which cause lytic infection of bacteria. Its simple regulatory apparatus is one of the most widely studied, serving as a model for genomes of more complex organisms. As mentioned above, previous nonlinear DNA studies [6–8, 10] have involved the T7 phage genome sequence. However, these studies focussed exclusively on the base sequence, with no consideration for the possible changing biological context of the information it contains. In fact the essentially linear processes of T7 DNA translocation and gene expression make this phage an excellent case study.

T7 is known to inject its double-stranded, linear DNA into a host *E. coli* cell in a stepwise, transcription-dependent manner [45]. The T7 genome contains 39,937 base pairs but initially only the first 850 of these base are translocated from the phage particle [46]. This initial fragment contains three strong promoters specific to *E. coli* RNAP $A_1 - A_3$ (in addition to the minor A_0 , or D , promoter with no known *in vivo* function) which initiate transcription of the phage sequence. The remainder of the genome is divided into three sections: The “early” region contains class I genes – those responsible for modifying host metabolism to favour phage production; the middle region, where class II genes govern phage replication; the “late region” of class III genes driving maturation and packaging of newly assembled phage DNA strands.

Transcription of the initial fragment serves a dual function: “pulling” downstream, early DNA from the phage particle into the host cell, in addition to transcribing the class I genes. The product of the first of these, gene 0.3, inactivates host defence (specifically type I restriction/modification) systems, therefore rapid recognition of a major promoter is vital for successful infection by wild-type T7. Another product of this early region is a T7 RNA polymerase, recognising its own specific promoters, which is responsible for transcribing the remainder of the genome, a process which proceeds in two steps: Entry of the middle region into the host cell is dependent upon the successful translation of class I genes. In turn, translocation of the late-transcribed region requires the products of early and mid-regions.

In contrast to gene expression in more complex organisms, there are very few “feedback” loops; indeed, a virtual simulation of the T7 life cycle has been developed [42]. There are two known loops which may have relevance to our analysis below: mid-late inhibition of class I (host-specific) promoters [43] and late inhibition of the mid (phage-specific) promoters [44].

2. The Model

At physiological temperatures the physically dominant mode of base-pair opening is the base-flipping, pendular oscillations of bases about their N-glycosidic bond in the mean base-pair plane. Such models previously considered for biological roles [6–11] are based upon the IFK [26] Hamiltonian:

$$\begin{aligned} \mathcal{H} = & \frac{1}{2} \sum_{i=1}^n I_i (\dot{\theta}_i^2 + \dot{\psi}_i^2) + \frac{1}{2} \sum_{i=1}^{n-1} (\kappa_i (\theta_{i+1} - \theta_i)^2 + \bar{\kappa}_i (\psi_{i+1} - \psi_i)^2) \\ & + \sum_{i=1}^n \sigma_i (1 - \cos(\theta_i - \psi_i)). \end{aligned} \quad (1)$$

Here θ_i , ψ_i are the angles of deflection of the i th base “pendulum” and that of its complement from equilibrium, while I_i is the inertial moment. Nearest-neighbour bases are coupled by an harmonic torsion potential with “stiffnesses” κ_i , $\bar{\kappa}_i$. Finally σ_i is the characteristic strength of the nonlinear H-bonding potential between complementary bases.

In earlier studies [6–8] homogeneous inertial moments and stiffness constants were assumed, with the only sequence-dependence residing in the H-bonding coupling constants σ_i . Specifically, for $i, 1 \dots, n$

$$I_i = I, \quad \kappa_i \equiv K = \bar{\kappa}_i.$$

In addition it was assumed that $\sigma_i = \lambda_i k$ where k is a generic coupling and λ_i takes the values 2 and 3 for A.T and G.C pairs respectively, accounting for the differing numbers of base pairs. With these approximations, one passes to angle sum- and difference-coordinates

$$\theta_i = \frac{1}{2}(u_i + v_i), \quad \psi_i = \frac{1}{2}(u_i - v_i).$$

The Hamiltonian thus obtained is

$$\begin{aligned} \mathcal{H}' = & \frac{1}{2} \sum_{i=1}^n I (\dot{u}_i^2 + \dot{v}_i^2) + \frac{K}{2} \sum_{i=1}^{n-1} (u_{i+1} - u_i)^2 + (v_{i+1} + v_i)^2 \\ & + \sum_{i=1}^n \lambda_i k (1 - \cos(u_i)). \end{aligned} \quad (2)$$

The equations of motion for the u_i reduce to the set of dimensionless, coupled equations:

$$\ddot{u}_i - (u_{i+1} - 2u_i + u_{i-1}) + \beta_i \sin u_i = 0; \quad u_i = \theta_i - \psi_i, \quad (3)$$

where the time variable has been rescaled, $t \rightarrow \sqrt{T/k}t$, and the parameter $\beta_i = \lambda_i k / K \equiv \lambda_i \eta$. To model the sequence variation as small perturbations to a homogenous solution, we first require the average value of the parameters β_i :

$$\beta = \left(2 \frac{n_{AT}}{n} + 3 \left(1 - \frac{n_{AT}}{n} \right) \right) \eta, \quad (4)$$

where there are n_{AT} occurrences of A.T pairs in the molecule. In a purely homogeneous approximation, $\beta_i \rightarrow \beta$, in the continuum limit the system of Eq. (3) reduces to the sine-Gordon equation,

$$\ddot{u} - u'' + \beta \sin u = 0, \quad (5)$$

which has a rich variety of solitonic solutions. A family of “breather” solutions of Eq. (5) with lengths L_μ and internal frequencies ω_μ is defined by

$$u_{br}(x, t) = 4 \tan^{-1} \left(\frac{\sin \omega_\mu t}{\omega_\mu L_\mu} \operatorname{sech} \left(\frac{x}{L_\mu} \right) \right), \quad (6)$$

where in terms of the classifying parameter μ

$$L_\mu = \beta^{-1/2} \operatorname{cosec} \mu, \quad \omega_\mu = \beta^{1/2} \cos \mu.$$

Note that the above relation imposes a minimum breather width and frequency which the model can support for a given set of environmental conditions. The smallness of β ensures that an approximate solution of the discrete, inhomogeneous model is of a similar form with slowly-varying parameters, thus our *ansatz* for Eq. (3) is

$$u_n = 4 \tan^{-1} \frac{\sin \omega t}{\omega L} \operatorname{sech} z_n; \quad z_n = (n - X)/L. \quad (7)$$

Here X is understood as a collective coordinate for the breather and for convenience we have omitted the μ subscript.

If the total energy is approximately conserved, upon substituting (7) into the Hamiltonian (2) one arrives at an expression for the effective potential in the collective coordinate X [8], associated with the propagation of the initial excitation on an inhomogenous background. We find, using the identity

$$1 - \cos u = 8/(\tan u/4 + \cot u/4)^2,$$

that the expression for the total energy takes the form

$$E(X; t) \equiv K(X; t) + V(X; t) + O(\beta^2) = 0. \quad (8)$$

Here

$$\begin{aligned}
K(X; t) &= \frac{8}{L^2} \sum_{i=1}^n D_i(X; t) \left(\alpha(t) \sinh^2 z_i (\dot{X})^2 \right. \\
&\quad \left. + \omega L \sqrt{\alpha(t) \left(\frac{1}{(\omega L)^2} - \alpha(t) \right)} \sinh 2z_i \dot{X} \right), \\
V(X; t) &= 8 \sum_{i=1}^n D_i(X; t) \left(\left(\frac{1}{L^2} - \omega^2 \alpha(t) \right) \cosh^2 z_i \right. \\
&\quad \left. + \alpha(t) \left(\frac{1}{L^2} \sinh^2 z_i + \beta_i \cosh^2 z_i \right) \right), \\
D_i(X; t) &= \frac{1}{(\alpha(t) + \cosh^2 z_i)^2}.
\end{aligned}$$

where the function $\alpha(t) \equiv (\sin(\omega t)/\omega L)^2$ governs the time-dependence of V . If the breather oscillation timescale is typically orders of magnitude smaller than that of its propagation along the DNA [41] we can replace the time-dependent potential by its average value [47]:

$$\begin{aligned}
V_{av}(X) &\equiv \frac{1}{T} \int_0^T V(X; t) dt \\
&= \frac{4}{L^2} \sum_{i=1}^n \frac{(\sec^2 \mu + \beta_i L^2 \tan^2 \mu) \cosh z_i}{(\tan^2 \mu + \cosh^2 z_i)^{3/2}}.
\end{aligned} \tag{9}$$

Owing to the nonlinear nature of the model the energy to translocate the initial deformation is several orders of magnitude less than that required to create the breather initially. We can derive a simple estimate of the “noisiness” of the sliding dynamics from the energy required to shift the breather by one base-pair:

$$\varepsilon(X) = \frac{K}{k_B T} (V_{av}(X_{i+1}) - V_{av}(X_i)) \tag{10}$$

where V_{av} is the time-averaged potential (9) and k_B is Boltzmann’s constant. For steep gradients the picture of sliding RNAP is thus analogous to a particle moving through an energy landscape while in flat regions it is more akin to a random walk.

3. Results

Having derived the breather effective potential (9) we compute the “landscape” corresponding to the T7 genome. It is natural, initially, to assume that the breather width is the size of a nonspecific RNAP complex. This size is not directly known,

either for *E. coli* or T7 RNAP. For certain other proteins the size of a nonspecific complex is estimated to be several times smaller [48] than that of a specific one. Therefore we assume upper bounds on L are provided by the DNA “footprint” size protected by RNAP in nuclease digestion experiments. For translocating *E. coli* and T7 elongation complexes these values are $L_B = 30$ bp [49] and $L_\phi = 24$ bp [50] respectively.

3.1. SEQUENCE ANALYSIS

For our initial sequence analysis we adopt model parameter values coinciding with those of Salerno's [6] original study of T7 promoters. Setting the ratio $\eta = 2 \times 10^{-3}$ implies that the lower bound for breather width is $L_{\min} \equiv \beta^{-1/2} \sim 15$ bp. Figure 1a shows the region of V_{av} corresponding to the initial 850 bp fragment of the T7 phage for a breather of width 30 bp. For comparison Figure 1c shows the time evolution of the system (3) with breathers initially placed at intervals within the fragment. Comparison of the three trajectories with the effective potential landscape in Figure 1a serves to verify that the direction and range of propagation agree for the two methods.

Now the σ^{70} subunit of *E. coli* holoenzyme RNAP recognises hexamers located 35 and 10 bases upstream of transcription initiation [51]. Many strong promoters

Figure 1. (a) Effective potential (9) for breathers in the initial T7 virion fragment. Initial binding sites for bacterial promoters are denoted by dots. (b) Noise parameter $\varepsilon(X)$ for the same sequence. (c) Evolution over 1000 time-steps of the system (3) with breathers initially placed at sites 460, 570 and 680.

Figure 2. Effective potential (9) for 30 bp wide breathers in the class I region of the T7 genome. Filled and unfilled dots denote respectively UP or -35 *E. coli* and $+1$ T7 promoter sites.

are enhanced by an additional element: contacts between the α subunit of an RNAP and an AT-rich sequence [52], an upstream (UP) element, centered approximately on sites -40 to -60 . Inspection of Figure 1a shows that the UP region of A_1 and the -35 sites for A_2 , A_3 (shown as dots) lie close to the bottom of potential wells: the respective initiation sites are 62, 29 and 21 bp upstream of these minima. Comparison with the noise parameter, ε plotted in Figure 1c shows that when the motion is strongly deterministic ($|\varepsilon| > 2$) it is invariably towards regions where promoter recognition can occur. The ε values in the initiation region of the strongest (A_1) bacterial promoter are 1.5 times greater than anywhere else in the T7 genome.

In fact there are seven *E. coli* RNAP specific promoters in the T7 genome, the first recognition sites for the six earliest are shown in Figure 2 as dots. The four minor (A_0 , B , C and E) promoters, while having no recognised *in vivo* function, were found to have initiation sites 61 (A_0 transcribes leftwards), 27, 28 and 17 bp downstream of deep minima. Figure 2 also shows the full class I region of the T7 genome, transcribed by the bacterial RNAP, extending from the 5' DNA end to the bacterial transcription terminator, TE. In the Genbank [54] reference sequence (accession number NC_001604) this corresponds to sites ~ 500 –7588. Note that other aspects of facilitated transport, such as dissociation followed by “hopping” or interdomain transfer, are likeliest to occur in locally flat regions, where the breather spends most time. In this way, the effect of multiple, broad-bottomed wells as kinetic “traps” might be minimised.

On the other hand, the deep minimum at approximately 6 Kb could be a desirable kinetic trap for the host RNAP as it lies 100 bp downstream of the gene coding for T7 RNA polymerase. The T7 RNAP initiates transcription at one of the two specific promoters (unfilled dots at the right side of Figure 2) and is thus responsible for

the subsequent internalisation and expression of the remainder of the T7 genome. This deep minimum thus represents the end of the region where the host RNAP is “useful”. One finds a similar, deep minimum at the class II/class III interface for a wide range of breather widths which could play a similar role, inhibiting late transcription from weaker class II promoters in favour of class III promoters.

3.2. PARAMETER VARIATION

Given the coarseness of the current model, it is important to understand how the results obtained may vary with respect to the parameter values. Up to an overall scaling, all parameter variation in the sequence-dependent part of (9) enter via the breather width, L , which governs sensitivity to sequence-dependent inhomogeneities: an increase of width leads to landscapes with fewer extrema which are also broader and larger in amplitude. The fundamental relationship governing effects of parameter variations is therefore $\text{cosec} \mu = L\sqrt{\beta}$. It is natural to associate the breather family parameter μ with the dimension of the protein DNA-interface and L with the “response” of the system for a given set of environmental conditions, encapsulated in η .

Understanding of model robustness is complicated by the way in which the sliding of RNAP changes. For example, if breathers do play a role in the location of T7 promoters $A_1 - A_3$ by bacterial RNAP then one might expect environmental changes which alter sliding behaviour to also influence promoter activity. It is known [55] that the activities of $A_1 - A_3$ are temperature dependent, with A_1 increasing from 20–37 °C while initiation at $A_{2,3}$ decreases under the same circumstances.

Due to decreased thermal stability, one expects a greater “reponse” of the helix to a deformation at increased temperature. For fixed μ this corresponds to an increase in L and decrease in η . The two graphs in Figure 3 are calculated for such circumstances with $L = 30$, $\eta = 0.002$ and $L = 67$, $\eta = 0.0004$ respectively. For the higher η value (on the left) a sliding RNAP is extremely likely to fall into one of the three wells associated with a major promoter.

Figure 3. Potential (9) computed for the T7 initial fragment for $\mu = \pi/6.05$. a) $\eta = 0.002$, $L = 30$ bp; b) $\eta = 0.0004$ ($L = 67$ bp). Dots denote, from left to right, UP and -35 sites for $A_1 - A_3$ bacterial promoters.

Figure 4. (a) Location, X , of minima of (9) nearest initiation sites of T7 phage promoter. The y-axis serves only to separate plotted points. (b) Scatter plot of initiation-downstream transcription unit distance (TU) versus initiation minima distance (Min).

Conversely, for the lower η value the well containing A_1 has greatly widened at the expense of the other two. Indeed, the -35 sites for A_2 , A_3 are now situated close to local maxima and the probability of an encounter with sliding RNAP would be significantly reduced. We note that minima close to one or more major promoter sites exist for a broad range of parameter values. One could argue that the overall sequence composition of the T7 initial fragment appears to confer some robustness of host promoter recognition against environmental variations.

Having outlined the qualitative variation of the system behaviour with parameter changes, we recompute the potential for $L_\phi = 24$ bp. From Figure 4a it is immediately seen that for none of the T7 promoters does the locally deepest minimum coincide with upstream, recognition sites. With replication origins ϕ_L and ϕ_R and the earliest phage promoters, $\phi_{1.1A}$, $\phi_{1.1B}$, omitted, minima appear to be correlated to the start of the first downstream coding sequence, as evidenced in Figure 4b.

4. Discussion

4.1. MODEL ASSUMPTIONS

The planar model of DNA presented is a highly simplified one, containing numerous assumptions which are unrealistic for modelling many DNA processes: There is no explicit allowance for the helical structure and its writhing/twisting behaviour. Many interactions with proteins involve major, localised conformational changes of DNA; however, the specific case of sliding RNAP may be an exception. Firstly, because such conformational changes are unlikely to be present immediately prior to closed complex formation [57] and secondly, there is some evidence that rates of RNAP sliding, under some conditions at least, are independent of supercoiling [32].

Another important assumption was the homogeneous, harmonic nature of the restoring torques. In fact it is known that simple, “base content” models of

helix-coil transition thermodynamics reproduce empirical data for short (≤ 15 bp) DNA oligonucleotides quite well [58, 59]. Specifically, encapsulating sequence dependence as AT and GC contents enables reproduction of such data at 310K in 1M NaCl solution (corrections due to change in salt concentration are discussed in [59]) with a mean (median) error of 9% (5%) (Bashford, J; unpublished). From our previous study of the thermodynamics of B-DNA helix-coil transition [60] we further estimate that the enthalpies of A.T and G.C pairs are in the ratio 1.56/3, which serves to enhance the distinction between the two types of base pair in Eq. (9). This accounts, in addition to differing numbers of H bonds, for the averaged effects of solution, neighbouring base-pairs and other interactions between the complementary pair.

The assumption of harmonicity for the stacking potential at large opening angles, however, is more questionable and should be further refined. Also, molecular calculations of the “base-flipping” in Watson-Crick pairs suggest [56] opening into the major groove is more energetically favoured for purine bases.

4.2. BREATHER DYNAMICS

The shape of the breather potential, used in the qualitative arguments above, depend only upon the ratios of $\eta = k/K$ and $\lambda_{A/T}/\lambda_{G/C}$. But physical properties of any breather depend on the actual parameter values. For example, the breather energy E and oscillation frequency ω may be derived as

$$E = \frac{16K}{L} \sim \sqrt{kK}, \quad (11)$$

$$\omega^2 = \frac{K}{I} \left(\beta - \frac{1}{L^2} \right). \quad (12)$$

Using the parameter values in Ref. [10]: $K = 5 \times 10^{-18}$ J, $I = 2 \times 10^{-43}$ kg m², in combination with our estimate based on data from Ref. [60]: $k = 1 \times 10^{-20}$ J, yields $\eta = 4.5 \times 10^{-3}$. Thus for a breather of width $L = 30$ bp we get

$$E \simeq 2.7 \times 10^{-18} \text{ J}, \quad \omega \simeq 1.0 \times 10^{12} \text{ s}^{-1}.$$

The energetic cost of creating this breather may be of the magnitude of the electrostatic attractions responsible for the nonspecific contact.

Concerning the size of the DNA helix deformation, we note that parameter μ provides an estimate of the amplitude of the base-pair opening. $u_{\max} = 4\mu$ when $\mu < \pi/4$. For $\beta = 0.0045$, as above, the amplitude for a 30 bp breather is $2\pi/3$, corresponding to individual pendulum deformations of 60° . This parameter set does not support breathers of width less than $\beta^{-1/2} \simeq 15$ bp. A variation of 20% in the value of K leads to maximum deformations of 52 – 65° : base pairs are bent but not

fully opened. These moderate conformational changes need not be incompatible with an anticipated absence of large deformations [57] accompanying nonspecific RNAP-DNA complexes.

The values for model parameters appearing in the literature are estimated from old experiments on DNA homopolymers, for example Refs. [62, 63] which is a difficult process. However the main results of our paper stem from (i) the *shape* of the potential (9) and (ii) the noise parameter, ε , defined by (10). For these two expressions changes in the parameter η can be offset by “tuning” the value of μ which is a relatively free parameter. The only potentially serious sensitivity is that of ε to large changes in k , the measure of dissociation energy for H-bonded base pairs. Fortunately, of the three parameters in (1), this is the most reliable quantity to estimate.

4.3. HELICAL MODEL

If the picture of sliding RNAP as a soliton-like deformation is subsequently shown to be incorrect, the correlations observed between potential minima and promoter sites still have to be explained. The soliton solutions of (1) preferentially move to AT-rich regions. Inspection of (9) shows the variation due to sequence is not linear in AT content, but a first “moment”, where the contribution from each base is weighted by its position relative to the central site X :

$$V_{\text{var}}(X) \sim \sum_i \beta_i w(z_i), \quad (13)$$

$$w(z) = \frac{\cosh z}{(\tan^2 \mu + \cosh^2 z)^{3/2}}.$$

Curiously, this weighting function coincides with the inverse radius of curvature for a hyperbolic curve $f(z) = \cosh z$. Such a term arises naturally in the Lorenz force experienced by a charged particle following a curved magnetic field line. Initially consider a particle of mass m , charge q , travelling along a uniform, straight magnetic field line. Its motion is determined by the Lorenz equation

$$\frac{d}{dt} \vec{v} = \frac{q}{m} \vec{v} \times \vec{B}.$$

Assuming the field line lies along the z axis, $\vec{B} = B\vec{e}_z$, the velocity equation is split into parallel and perpendicular components

$$\begin{aligned} \frac{d}{dt} v_{\parallel} &= 0, \\ \frac{d}{dt} \vec{v}_{\perp} &= \frac{qB}{m} \vec{v}_{\perp} \times \vec{e}_z \end{aligned}$$

The general solution to these equations is a helical trajectory, with time-dependent coordinates

$$\begin{aligned}x(t) &= x_0 + \frac{|v_\perp|}{\omega} \sin(\omega t + \phi), \\y(t) &= y_0 + \frac{|v_\perp|}{\omega} \cos(\omega t + \phi), \\z(t) &= z_0 + v_\parallel t,\end{aligned}$$

where (x_0, y_0, z_0) denotes the initial location of the particle and ω determines the helical frequency. This problem naturally resembles the electrostatic sliding of a protein “particle” along the grooves of the DNA helix. Here the role of gyro frequency is played by the twist of the helix, while the guiding centre of particle motion $(x_0, y_0, z(t))$ corresponds to the central helical axis of the DNA.

Consider now the effect of introducing a curve into the helical axis: a particle travelling along a curved field line experiences a centrifugal force upon its guiding centre. In a local coordinate system this is

$$\frac{mv_\parallel^2}{|r_c(s)|} \frac{\vec{r}_c(s)}{|r_c(s)|}$$

where $|r_c|$ and s denote the radius of curvature and line element along the field line. Similarly let us here write an analogous expression

$$\vec{F}_c = \frac{\mathcal{E}}{r_c} \vec{r}_c \quad (14)$$

where the quantity $\mathcal{E}$ has the dimensions of energy. In particular, we assume that locally the bend can be approximated by $z(\xi) = \cosh \xi$. Then, c.f. (13),

$$|\vec{F}_c(\xi)| = \mathcal{E} \frac{\cosh \xi}{(1 + \sinh^2 \xi)^{3/2}}. \quad (15)$$

It follows that in the continuum limit the time-averaged breather potential could also be thought of as the work done by a “centrifugal force” on a sliding RNAP as it navigates a bend in the helix. Therefore the “potential” (9) can conceivably be arrived at via simple considerations of thermal stability (in a planar model) or bending deformations (in a helical model), two of the most commonly suggested mechanisms for enhancing promoter recognition.

4.4. SUPERHELICITY

A mechanism of localised DNA deformation with demonstrated biological significance [18, 65, 66] is that of superhelical stress-induced DNA denaturation (SSID).

Roles for SSID in gene regulation have been proposed [18] in regard to both open complex formation and transcription. In the former instance, promoter sites are easily destabilized by superhelical stress. In the latter, the action of local helix unwinding by transcribing RNAP results in waves of positive (negative) superhelicity propagating downstream (upstream) of the transcription complex. Computation of SSID profiles indicates [18, 66] AT rich regions (down-) up-stream of the (3') 5' ends of transcription units are prone to localised over/under-winding acting as a possible “sink” for propagating superhelicity and ensuring smooth transcription.

The breather potential (9), which also picks out regions of AT, shows that transcription units of at least 10^3 bp in length are often demarcated by minima, in agreement with the above observations. This is especially the case for the 3' ends of T7 genes 1 and 6, the last genes in class I and II regions respectively. In these instances the AT richness may also confer extra flexibility, making these suitable pause sites in the stepwise internalisation of the phage genome, or as mentioned above act as a kinetic trap, used in inhibiting class I or II transcription.

4.5. CORRELATIONS

In reporting promoter-extrema correlations two points should be kept in mind. Firstly, the assumed breather widths coincide with the sizes of the elongation RNAP-DNA complexes. Therefore potential minima could be indicative of deformation associated with transcription, as appears to be the case for T7 phage promoters, shown in Figure 4. Regarding nonspecific complexes, the values $L_B = 30$ and $L_\phi = 24$ bp should be considered as upper bounds for an experimentally undetermined quantity. The correlations reported in this study persist for the ranges $20 \leq L_B \leq 30$ and $18 \leq L_\phi \leq 24$. For sizes less than 18 bp, the increasing roughness of Eq. (9) causes difficulty in identifying correlations.

The second caveat is that only correlations between promoter initiation and the deepest local minimum have been considered. For some T7 promoters shallow upstream wells also exist. Moreover the effect of thermal noise has not been considered. Only with full dynamical simulations can connections between the local topography of Eq. (9) and facilitated target location be properly studied.

It is difficult to see how kink solutions of the planar model (1), previously considered [6–11], might mimic physical profiles of base-pair opening. Kinks will also move preferentially to AT rich regions, presumably the reason why promoter sequences A_1 [6], A_3 and A_0 [7] were concluded to be “dynamically active”. The unit-mass potential for kinks, initially at rest, moving in a slowly-varying background was derived by Salerno and Kivshar [8]. The sequence variation is contained in a term analogous to (13), however the weighting function is

$$W_k(z) = \text{sech}^2 z.$$

This coincides with the breather function for small $\tan^2 \mu$, illustrating why similar results for the major T7 promoter sequences are obtained for both kink [6–8] and breather solitons.

5. Conclusion

In this paper we have re-examined Salerno's nonlinear DNA model, postulating a role for localised soliton excitations in approximating the sliding component of facilitated target location of RNA polymerase. We found that such deformations would involve moderate bending of individual base pairs and that their energy of translocation is consistent with a picture of noisy, deterministic dynamics. Both of these observations are also consistent with current, limited knowledge of RNAP sliding and nonspecific complexes. A qualitative correspondence of these solitons and localised bending in a helical model was also demonstrated.

The dynamical picture of sliding which emerged also suggests that the random/deterministic nature of the motion is sequence-dependent, with translocation in relatively homogeneous regions being effectively random. The corollary, that interplay between adjacent random and deterministic regions could constitute a search “algorithm”, is speculative and, we believe, merits further investigation.

Our analysis of the T7 genome showed good correlations between AT-rich regions and the recognition sites of host-specific promoters used for early phage transcription. For phage-specific promoters, regions of maximal AT-richness correlated with the start of the coding sequence immediately downstream. As discussed above this may be connected with transcription and while there is no obvious correlation with recognition sites, a full description of facilitated target location needs to account for the thermal background. This is a subject of current investigation.

We note that there has been suggestion [64] that virion proteins injected into the host cell with the initial T7 fragment may i) inhibit the nonspecific binding of restriction enzymes and other proteins to DNA; ii) have an affinity for *E. coli* RNAP, negating the requirement for direct promoter recognition *in vivo*. Similarly, inhibition of class I and II transcription is known to be performed by T7 gene products: kinase (gene 0.7) and lysozyme (gene 3.5) respectively.

However we see similar correlations for the UP and σ^{70} sites of bacterial promoters in other members of the T7 viral supergroup, in addition to genomes of the unrelated phages T4 and T5 (see Figure 5). This may be suggestive of a mechanism at work to enhance promoter recognition/inhibition in lytic phage genomes, although in the presence of functional proteins this mechanism can be relegated to an auxiliary role, such as in T7.

It is important to investigate whether planar base-flipping/helical bending deformation patterns can be used to simulate protein-DNA interactions in DNA sequence analysis. The correlations reported here, to our knowledge for the first time, could have been made via other “nonlinear” analyses of AT content, had a motivation

Figure 5. Representative region of T5 genome potential, showing correlations between potential minima and -35 sites for *E. coli* promoters ($L = 30$ bp).

been apparent. Propagation of breathers in a non-linear, toy model of DNA provide a source for such motivation. It may be that herein lies the true value of a model with such a controversial history.

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References

1. Peyrard, M.: Nonlinear Dynamics and Statistical Physics of DNA, *Nonlinearity* **17** (2004), R1–R40.
2. Gaeta, G.: Results and Limitations of the Soliton Theory of DNA Transcription, *J. Biol. Phys.* **24** (1999), 81–96.
3. Peyrard, M. and Bishop, A.R.: Statistical Mechanics of a Nonlinear Model for DNA Denaturation, *Phys. Rev. Lett.* **62** (1989), 2755–2758.
4. Yakushevich, L.V.: Is DNA a Nonlinear Dynamical System where Solitary Conformational Waves are Possible?, *J. Biosci.* **26** (2001), 305–313.
5. Bruant, N., Flatters, D., Lavery, R. and Genest, D.: From Atomic to Mesoscopic Descriptions of the Internal Dynamics of DNA, *Biophys. J.* **77** (1999), 2366–2376.
6. Salerno, M.: Discrete Model for DNA-Promoter Dynamics, *Phys. Rev. A* **44** (1991), 5292–5297.
7. Salerno, M.: Dynamical Properties of DNA Promoters, *Phys. Lett. A* **167** (1992), 49–53.

8. Salerno, M. and Kivshar, Yu.S.: DNA Promoters and Nonlinear Dynamics, *Phys. Lett. A* **193** (1994), 263–266.
9. Salerno, M.: Nonlinear Dynamics of Plasmid PBR322 Promoter, Chapter 10 in M. Peyrard (ed.), *Nonlinear Excitations in Biomolecules*, Edition de Physique, Springer, New York (1995).
10. Lennholm, E. and Hörnquist, M.: Revisiting Salerno's Sine-Gordon Model of DNA, Active Regions and Robustness, *Physica D* **177** (2003), 233–241.
11. Cuenda, S. and Sánchez, A.: Disorder and Fluctuations in Nonlinear Excitations in DNA, *Fluct. Noise Lett.* **4** (2004), L491–L504.
12. Englander, S.W. *et al.*: Nature of the Open State in Long Polynucleotide Double Helices, Possibility of Soliton excitations, *Proc. Natl. Acad. Sci.* **77** (1980), 7222–7226.
13. Gaeta, G., Reiss, C., Peyrard, M. and Dauxios, T.: Simple Models of Nonlinear DNA Dynamics, *Riv. del. Nuov. Cim.* **17** (1994), 1–48.
14. Gabriel, C. *et al.*: Microwave Absorption in Aqueous Solutions of DNA, *Nature* **328** (1987), 145–146.
15. Bigio, I.J., Gosnell, T.R., Mukherjee, P. and Safer, J.D.: Microwave Absorption Spectroscopy of DNA, *Biopolymers* **33** (1993), 147–150.
16. Guéron, M., Kochoyan, M. and Leroy, J.L.: A Single Mode of DNA Base-Pair Opening Drives Imino Proton Exchange, *Nature* **328** (1987), 89–92.
17. Frank-Kamenetskii, M.: Physicists Retreat Again, *Nature* **328** (1987), 108.
18. Benham, C.J.: Duplex Destabilization in Superhelical DNA is Predicted to Occur at Specific Transcriptional Regulatory Regions, *J. Mol. Biol.* **255** (1996), 425–434.
19. Choi, C.H. *et al.*: DNA Dynamically Directs its Own Transcription Initiation, *Nucl. Acids. Res.* **32** (2004), 1584–1590.
20. Kanhere, A. and Bansal, K.: A Novel Method for Prokaryotic Promoter Prediction Based on DNA Stability, *BMC Bioinformatics* **6** (2005), 1–10.
21. Bolshoy, A., McNamara, P., Harrington, R.E. and Trifonov, E.: Curved DNA Without A-A: Experimental Estimation of All 16 DNA Wedge Angles, *Proc. Natl. Acad. Sci.* **88** (1991), 2312–2316.
22. Scipioni, A., Anselmi, C., Zuccheri, G., Samori, B. and De Santis, P.: Sequence-Dependent DNA Curvature and Flexibility from Scanning Force Microscopy Images, *Biophys. J.* **83** (2002), 2408–2418.
23. Lankas, F.: DNA Sequence-Dependent Deformability – Insights from Computer Simulations, *Biopolymers* **73** (2004), 327–339.
24. Ponomarev, S.Y., Thayer, K.M. and Beveridge, D.L.: Ion Motions in Molecular Dynamics Simulations on DNA, *Proc. Natl. Acad. Sci.* **101** (2005), 14771–14775.
25. Polozov, R.V. *et al.*: Electrostatic Potentials of DNA. Comparative Analysis of Promoter and Nonpromoter Sequences, *J. Biomol. Struct. Dyn.* **16** (1999), 1135–1143.
26. Braun, O.M. and Kivshar, Yu.S.: *The Frenkel-Kontorova Model: Concepts, Methods and Applications*, Springer, Berlin, 2004.
27. von Hippel, P.H. and Berg, O.G.: Facilitated Target Location in Biological Systems, *J. Biol. Chem.* **264** (1989), 675–678.
28. Fickert, R. and Müllerhill, B.: How lac Repressor Finds lac Operator *in vivo*, *J. Mol. Biol.* **226** (1992), 59–68.
29. Jiao, Y., Cherny, D.I., Heim, G., Jovin, T.M. and Schäffer, T.E.: Dynamic Interactions of p53 with DNA in Solution by Time-Lapse Atomic Force Microscopy, *J. Mol. Biol.* **314** (2001), 233–243.
30. Park, C.S., Wu, F.Y.H. and Wu, C.S.: Molecular Mechanism of Promoter Selection in Gene Transcription, *J. Biol. Chem.* **257** (1982), 6950–6956.
31. Singer, P.T. and Wu, C.S.: Kinetics of Promoter Search by *Escherichia Coli* RNA Polymerase, *J. Biol. Chem.* **263** (1988), 4208–4214.
32. Smeekins, S.P. and Romano, L.J.: Promoter and Nonspecific DNA Binding by the T7 RNA Polymerase, *Nucl. Acids. Res.* **14** (1986), 2811–2827.

33. Kabata, H. *et al.*: Visualisation of Single Molecules of RNA Polymerase Sliding Along DNA, *Science* **262** (1993), 1561–1563.
34. Guthold, M. *et al.*: Direct Observation of One-Dimensional Diffusion and Transcription by *Escherichia coli* RNA Polymerase, *Biophys. J.* **77** (1999), 2284–2294.
35. Jeltsch, A. and Pingoud, A.: Kinetic Characterisation of Linear Diffusion of the Restriction Endonuclease *EcoRV* on DNA, *Biochemistry* **97** (1998), 2160–2169.
36. Nardone, G., George, J. and Chirikjian, J.G.: Differences in the Kinetic Properties of BamHI Endonuclease and Methylase with Linear DNA Substrates, *J. Biol. Chem.* **261** (1986), 2128–2133.
37. Berg, O.G., Winter, R.B. and von Hippel, P.H.: Diffusion-Driven Mechanisms of Protein Translocation on Nucleic Acids. 1. Models and Theory, *Biochemistry* **20** (1981), 6929–6948.
38. Barbi, M., Place, C., Popkov, V. and Salerno, M.: A Model of Sequence-Dependent Protein Diffusion Along DNA, *J. Biol. Phys.* **30** (2004), 203–226.
39. Sataric, M.V. and Tuszyński, J.A.: Impact of Regulatory Proteins on the Nonlinear Dynamics of DNA, *Phys. Rev. E* **65** (2002), 1901–1911.
40. Ting, J.J.-L. and Peyrard, M.: Effective Breather-Trapping Mechanism for DNA Transcription, *Phys. Rev. E* **53** (1996), 1011–1018.
41. Ting, J.J.-L.: DNA Transcription Mechanism with a Moving Enzyme, *Intl. J. Mod. Phys. A* **7** (1997), 1125–1132.
42. Endy, D., You, L., Yin, J. and Molineux, I.J.: Computation, Predictions and Experimental Tests of Fitness for Bacteriophage T7 Mutants with Permuted Genomes, *Proc. Natl. Acad. Sci.* **97** (2000), 5375–5380.
43. Hesselbach, B.A. and Nakada, D.: Host Shutoff Function of Bacteriophage T7: Involvement of T7 Gene 2 and Gene 0.7 in the Inactivation of *Escherichia coli* RNA Polymerase, *J. Virol* **24** (1977), 736–745.
44. Moffat, B.A. and Studier, F.W.: T7 Lysozyme Inhibits Transcription by T7 RNA Polymerase, *Cell* **49** (1987), 221–227.
45. Zavriev, S.K. and Shemyakin, M.F.: RNA Polymerase-Dependent Mechanism for the Stepwise T7 Phage DNA Transport from the Virion into *E. coli*, *Nucl. Acids. Res.* **10** (1982), 1635–1652.
46. Garcia, L.R. and Molineux, I.J.: Rate of Translocation of Bacteriophage T7 DNA Across the Membranes of *Escherichia coli*, *J. Bacteriol.* **177** (1995), 4066–4076.
47. Zhang, F.: Breather Scattering by Impurities in the Sine-Gordon Model, *Phys. Rev. E* **58** (1998), 2558–2563.
48. Tsodikov, O.V., Holbrook, J.A., Shkel, I.A. and Record, M.T. Jr.: Analytic Binding Isotherms Describing Competitive Interactions of a Protein Ligand with Specific and Nonspecific Sites on the Same DNA Oligomer, *Biophys. J.* **81** (2001), 1960–1969.
49. von Hippel, P.H.: An Integrated Model of the Transcription Complex in Elongation, Termination and Editing, *Science* **281** (1998), 660–665.
50. Imburgio, D., Rong, K.Ma. and McAllister, W.T.: Studies of Promoter Recognition and Start Site Selection by T7 RNA Polymerase Using a Comprehensive Collection of Promoter Variants, *Biochemistry* **39** (2000), 10419–10430.
51. Mulligan, M.E., Hawley, D.K., Entriken, R. and McClure, W.R.: *Escherichia Coli* Promoter Sequences Predict *in vitro* RNA Polymerase Selectivity, *Nucleic. Acids. Res.* **12** (1984), 789–800.
52. Estrem, S.T. *et al.*: Bacterial Promoter Architecture: Subsite Structure of UP Elements and Interactions with the Carboxy-Terminal Domain of the RNA Polymerase α Subunit, *Genes Dev.* **13** (1999), 2134–2147.
53. Sclavi, B. *et al.*: Real-Time Characterisation of Intermediates in the Pathway to Open Complex Formation by *Escherichia coli* RNA Polymerase at the T7A1 Promoter, *Proc. Natl. Acad. Sci.* **102** (2005), 4706–4711.
54. National Center for Biotechnology Information Website. <http://www.ncbi.nlm.nih.gov/Entrez>.

55. Dausse, J.P., Sentenac, A. and Fromageot, P.: Interaction of RNA Polymerase from *Escherichia coli* with DNA. Effect of Temperature and Ionic Strength on Selection of T7 DNA Early Promoters, *Eur. J. Biochem* **65** (1976), 387–393.
56. Giudice, E., Várnai, P. and Lavery, R.: Base-Pair Opening Within B-DNA: Free Energy Pathways for GC and AT Pairs from Umbrella Sampling Situations, *Nucl. Acids. Res.* **31** (2003), 1434–1443.
57. Murakami, K.S., Masuda, S. and Darst, S.A.: Structural Basis of Transcription Initiation: RNA Polymerase Holoenzyme at 4 Å Resolution, *Science* **296** (2002), 1280–1284.
58. Frank-Kamanetskii, M.: Simplification of the Empirical Relationship Between Melting DNA, its GC Content and Concentration of Sodium ions in Solution, *Biopolymers* **10** (1971), 2623–2624.
59. SantaLucia, J. Jr.: A Unified View of Polymer, Dumbbell and Oligonucleotide DNA Nearest-Neighbour Thermodynamics, *Proc. Natl. Acad. Sci.* **95** (1998), 1460–1465.
60. Bashford, J.D. and Jarvis, P.D.: A Base-Pairing Model of Duplex Formation I: Watson-Crick Pairing Geometries, *Biopolymers* **78** (2005), 287–297.
61. SantaLucia, J. Jr., Allawi, H.T. and Seneviratne, P.A.: Improved Nearest-Neighbour Parameters for Predicting DNA Duplex Stability, *Biochemistry* **35** (1996), 3555–3562.
62. Yakushevich, L.V.: Scattering of Neutrons and Light by DNA Solitons, *Stud. Biophys.* **103** (1984), 171–178.
63. Yakushevich, L.V.: The Effects of Damping, External Fields and Inhomogeneity on the Nonlinear Dynamics of Biopolymers, *Stud. Biophys.* **121** (1987), 201–207.
64. Molineux, I.J.: No Syringes Please, Ejection of Phage T7 DNA from the Virion is Enzyme Driven, *Mol. Microbiol.* **40** (2001), 1–8.
65. Benham, C.J.: Sites of Predicted Stress-Induced DNA Duplex Destabilization Occur Preferentially at Regulatory Regions, *Proc. Natl. Acad. Sci.* **90** (1993), 2999–3003.
66. Wang, H., Noordewier, M. and Benham, C.J.: Stress-Induced DNA Duplex Destabilization (SIDD) in the *E. coli* Genome: SIDD Sites are Closely Associated with Promoters, *Genome Research* **14** (2004), 1575–1584.