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# DNA dynamics play a role as a basal transcription factor in the positioning and regulation of gene transcription initiation

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## ABSTRACT

**We assess the role of DNA breathing dynamics as a determinant of promoter strength and transcription start site (TSS) location. We compare DNA Langevin dynamic profiles of representative gene promoters, calculated with the extended non-linear PBD model of DNA with experimental data on transcription factor binding and transcriptional activity. Our results demonstrate that DNA dynamic activity at the TSS can be suppressed by mutations that do not affect basal transcription factor binding–DNA contacts. We use this effect to establish the separate contributions of transcription factor binding and DNA dynamics to transcriptional activity. Our results argue against a purely ‘transcription factor-centric’ view of transcription initiation, suggesting that both DNA dynamics and transcription factor binding are necessary conditions for transcription initiation.**

## INTRODUCTION

RNA polymerases require access to a locally denatured single-strand DNA segment (2,3) at the transcription start site (TSS) in order to initiate transcription. It has been demonstrated that introduction of an artificial bubble at the TSS of a viral promoter, via insertion of a 5-bp mismatched segment, is sufficient for the polymerase to initiate transcription in the absence of basal transcription factors (4,5). Use of a negatively supercoiled DNA template (5–8) can also obviate the requirement by polymerase II for basal transcription factors binding (5–7) and helicase activity (8). It has been suggested that under natural conditions *in vivo*, formation of the

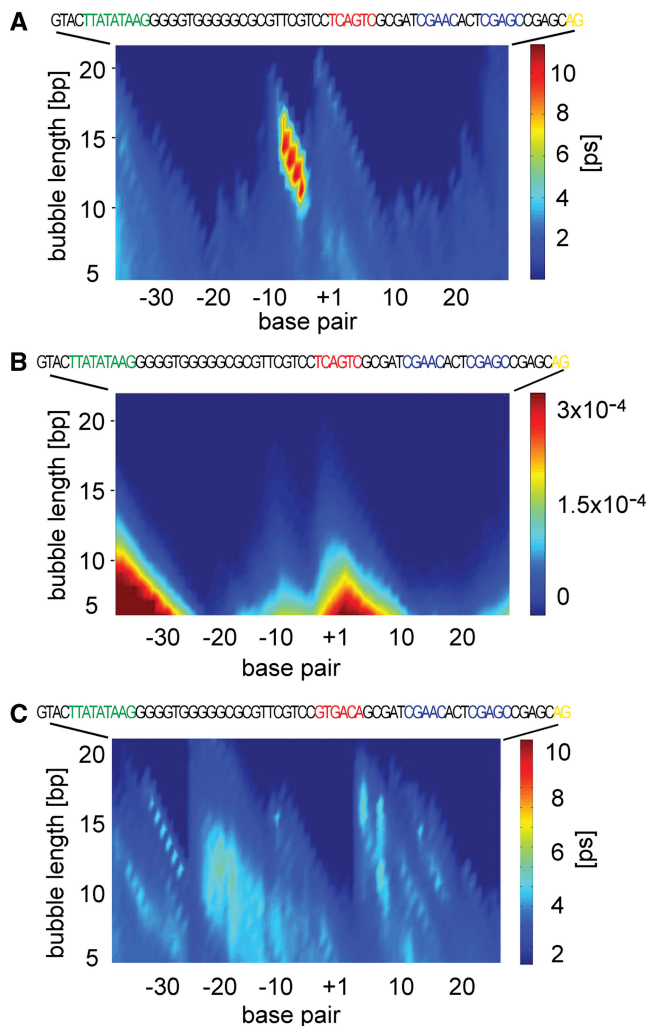
transcriptional bubble is seeded by transient, thermally induced strand separation motions of the DNA double helix, commonly known as DNA breathing (9). To investigate this possibility, we have been studying the sequence dependence of breathing dynamics with the non-linear Peyrard–Bishop–Dauxois model (PBD) of DNA (10,11). In support of a link between spontaneous DNA strand separation and transcription initiation, we found that mammalian promoter sequences frequently exhibit a breathing dynamics maximum (bubble) coinciding with the TSS (4,9). We introduced the use of Langevin molecular dynamic (LMD) simulations and use of three dynamic criteria: bubble length, bubble amplitude and bubble lifetime, which can be extracted from the simulated dynamic trajectories of experimentally identified TSS (4). Bubble length is defined as the number of consecutive base pairs that are simultaneously separated from their hydrogen bond minima by more than a given distance threshold (the bubble amplitude). Simulations of several mammalian core promoters demonstrated that a relatively large (length: ~10 bp; amplitude  $A$ : >1.5 Å) and stable (lifetime  $\tau$ : >5 ps) bubble forms frequently at the examined TSS (4). We reported that A/T-rich regions such as TATA boxes exhibit faster, lower amplitude motions than TSS regions (4,12). G/C-rich promoters, however, display less obvious bubble-forming motifs in the simulations (4).

The main source of structural and dynamic heterogeneity in G/C-rich sequences presumably originates from a dramatic difference in the stacking interaction between GG/CC steps on the one hand and CG/CG and GC/GC on the other (1,13,14). However, the original PBD Hamiltonian does not account for the sequence dependence of the stacking potentials and performs poorly at reproducing the melting transitions of G/C-rich DNA. For accurate analysis of G/C-rich DNA, we recently

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.





**Figure 1.** LMDs simulations of the collective DNA openings in the SCP1 'Super promoter' predict two major loci of dynamic activity. (A) Average lifetime with length  $L$  (vertical axis), beginning at a given nucleotide position (horizontal axis) relative to the TSS ('+1'). (B) Probability for the formation of bubbles of amplitude  $>3.5$  Å. (C) Average bubble lifetimes of a transcriptionally inactive SCP1 variant mutated at the Inr sequence (16). The SCP1 promoter element motifs are shown at the top. Promoter element motifs are indicated with colored boxes: Inr, red; MTE, blue; DPE, yellow.

content and the effect of adjacent sequences. Remarkably, the m1SCP1 mutant, which differs from the wild-type sequence by four-point mutations located *outside* of the TSS, exhibits a dramatically different dynamic profile (Figure 2A). The mutations suppress the dynamic activity of the TSS, clearly silencing the TSS bubble, while preserving the sequence. The second mutant, m2SCP1 displayed a dynamic profile essentially identical to the wild type (Figure 2A). In both mutants, the Inr, MTE and DPE motif sequences were preserved.

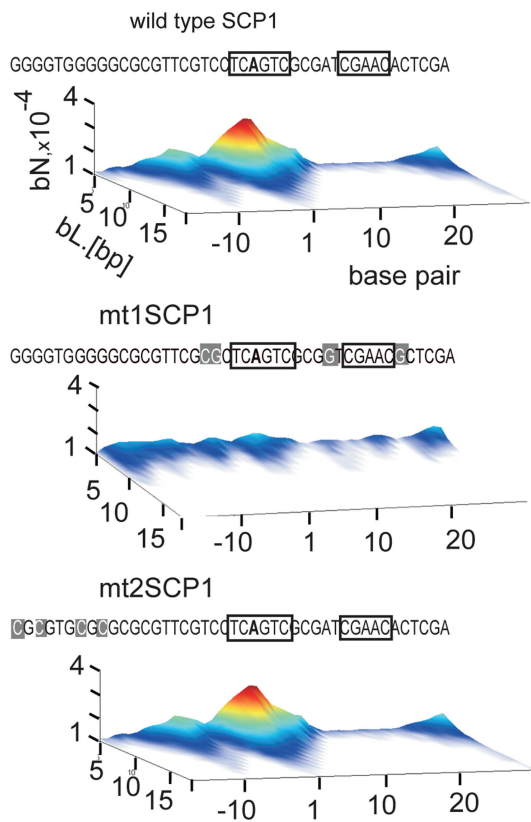
The role of the Inr sequence element in transcription is generally attributed to binding of transcriptional initiator factors such as the large TFIID complex (16,19) and/or YY1 (5). These proteins serve to recruit the polymerase and the other basal transcription factors at the TSS (6,8). The m1SCP1 sequence retains the original Inr element,

but lacks the characteristic TSS dynamic signature, providing a test case to establish whether binding of the basal transcription factors is *sufficient* for transcription, or whether the TSS dynamic signature is *necessary*. To confirm that transcription factor binding was, as intended, unaffected by the m1SCP1 mutations, we performed gel shift assay with TFIID and basal transcription factors (Figure 2B). As positive control, we conducted gel shift reactions with the wtSCP1 promoter oligo fragment. Reactions were assembled with equal protein amounts of TFIID (3 ng/reaction) and TFIIB (2 ng/reaction) alone and together with transcription factors TFIIF (4 ng), TFIIE (3 ng) and TFIIA (3 ng). The results suggest that both the wild-type and the m1SCP1 oligos form nearly identical complexes with TFIID and the tested basal transcription factors. The observed complexes result from sequence-specific recognition, since presence of unlabeled wtSCP1 oligo in the reactions competes equally well for protein binding with both radioactively labeled wtSCP1 and m1SCP1 fragments. To verify the composition of the protein–DNA complexes in crude nuclear extract, we performed gel shift reactions with HeLa extract and anti-TFIIF basal transcription factor-specific antibodies (Supplementary Data). The results suggest that the selected mutations in m1SCP1 only result in suppression of bubble dynamics at the TSS without affecting the binding of the basal pre-initiation transcription complex. The m2SCP1 promoter variant displays both intact dynamics and protein binding (data not shown).

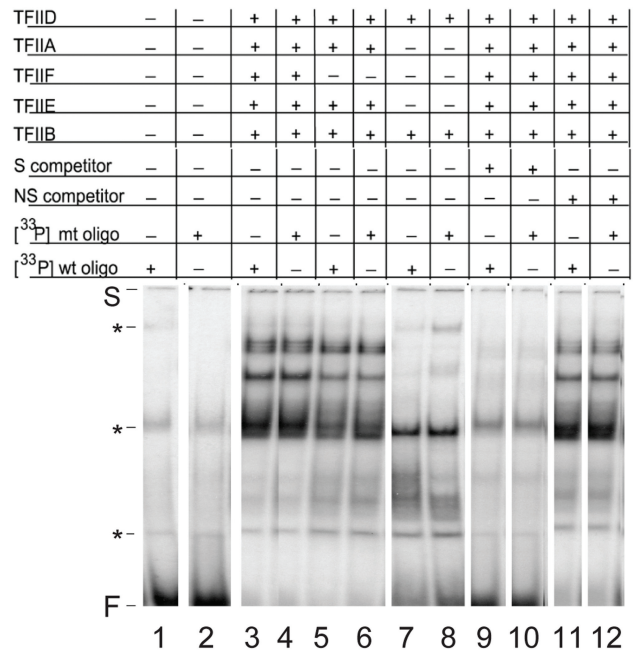
The effect of the mutations on promoter strength was assessed by transiently transfecting wtSCP1, m1SCP1 and m2SCP1 promoter templates in HeLa cells. The transcriptional activity of the promoter variants was determined by measuring the cellular levels of specific RNA transcripts in real-time PCR reactions (Q-PCR). As expected, wtSCP1 and m2SCP1 support high level of transcription in HeLa cells resulting in accumulation of specific RNA (Figure 2C). In comparison, m1SCP1 showed a 4-fold decrease in the level of RNA transcripts. The results of these experiments suggest that suppression of TSS bubble dynamics leads to a decrease in promoter activity, independent of basal transcription factor binding to the core promoter.

To further establish the requirement for strong DNA dynamics in determining a TSS, we conducted EPBD Langevin dynamic simulations on the mouse TS promoter (17,20). The TS promoter is a CpG island promoter that does not contain any of the known elements present in SCP1. It has been suggested that CpG island promoters are commonly associated with constitutively expressed housekeeping genes and may be regulated differently than the other known classes of promoters, such as promoters containing the TATA and Inr elements. TS is a 'dispersed' promoter (19,21), by virtue of having multiple TSS dispersed over a 100-bp region (17). This is in contrast to 'focused' promoters such as SCP1, which display one or several clearly defined start sites. Most of the TSSs of the TS promoter are known to be regulated by the Ets family of transcription factors in collaboration with Sp1 (20).

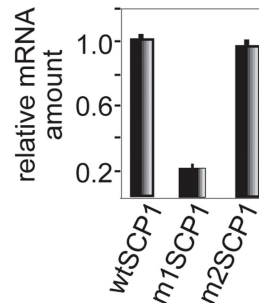
**A** EPBD simulations - bubble probability profile



**B** Gel shift



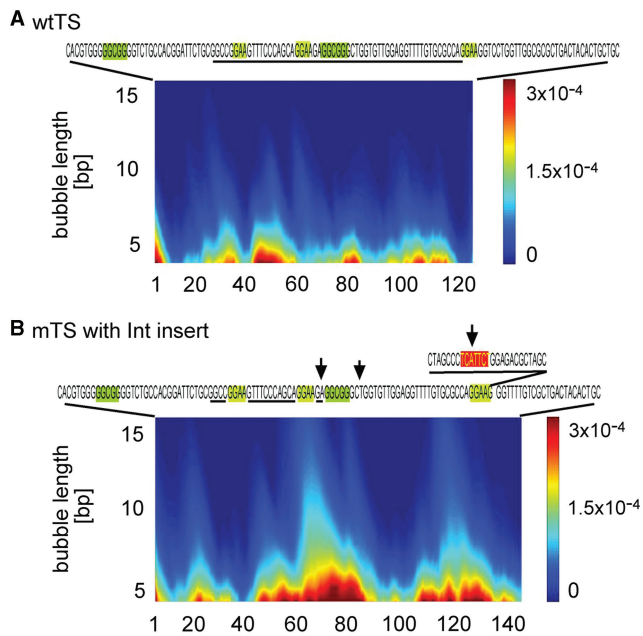
**C** transcription in cells



**Figure 2.** EPBD-derived mutations that change bubble probability profile and transcriptional activity while preserving TFIID complex formation at the SCP1 promoter. (A) Bubble probability profiles of the wild-type SCP1 promoter (wtSCP1), m1SCP1, m2SCP1 mutant variants designed to silence transcription activity without affecting protein-binding sites. The probability (*z*-axis) for the formation of bubbles of amplitude >3.5 Å with length *L* (*y*-axis) beginning at a given nucleotide position (*x*-axis) relative to the TSS (+1'). The wtSCP1, m1SCP1 and m2SCP1 sequences are shown at the top. Mutated residues are indicated with gray boxes. Protein binding sites are indicated with black frames. The profile of m2SCP1 is identical to wild type SCP1 as shown at the bottom. (B) Gel shift reactions. Effect of the m1SCP1 mutations on complex formation between TFIID, TFIIB, TFIIF, TFIIE and the Inr promoter fragment. Band shift reactions received a <sup>33</sup>P-labeled, double-stranded oligonucleotide (0.12 nM) containing the wild-type (lanes 1, 3, 5, 7, 9 and 11) and the m1SCP1 Inr box sequence (lanes 2, 4, 6, 8, 10 and 12), as indicated in (A). Transcription factors samples are as follows: lanes 1 and 2 bovine serum albumin; lanes 3–12 received equal amounts (in micrograms) of transcription factors as indicated above the lanes. The reactions in lanes 9 and 10 received 3 nM of homologous wild-type cold SCP1 oligonucleotide as a competitor. The reactions in lanes 11 and 12 received 10 nM of unrelated cold oligonucleotide as a competitor. The presence (+) or absence (–) of competitor oligo DNA and basal transcription factors in the reactions is indicated above the lanes. The positions of the gel shift start (*S*), the free DNA (*F*) and the non-specific gel shift products (asterisk) are indicated. (C) Transient cell transfection experiments were carried out to measure wtSCP1, m1SCP1 and m2SCP1 promoter activity. The three pUC119-based constructs (16) (2 μg/10<sup>6</sup> cells) were transfected by electroporation into HeLa cells. Total RNA was extracted from the cells and subject to Q-PCR-based analysis with pUC119 primers to measure cellular level of promoter-specific RNA transcripts. To ensure equal transfection efficiency, DNA instead of RNA was extracted from an aliquot from each reaction, and subjected to Q-PCR with the same primers. Data are expressed as fold induction relative to wtSCP1 mRNA level (on the vertical); all values are normalized to the cellular reference gene ARPO0 mRNA level of expression; reactions were run in triplicate; results were consistent in four independent experiments; error bars, mean ± SD, *n* = 4. The normalized values are plotted as a bar graph and the identity of the promoter-specific transcripts level is shown below the bars.

The simulations of the wild-type TS promoter revealed a dynamic activity that is evenly distributed and noticeably weaker than SCP1 (Figure 3A). Conspicuously lacking is the characteristic long-lived bubbles observed

for SCP1 (Figure 2A) and other previously studied ‘focused’ promoters (4). This result is again consistent with the notion that a relatively stable, well-defined bubble is required to define a strong, localized TSS.



**Figure 3.** Changes in the TS promoter bubble probability profile in response to mutations. (A) Wild-type TS promoter (wtTS). The promoter sequence is shown at the top. Experimentally determined window with several TSSs (17) is underlined. (B) Mutant promoter variant (mTS) with altered TSS distribution due to the insertion of an Int-containing sequence (17). The exact position of the insertion is shown above the mTS sequence. The Int is indicated by a red box. The mTS promoter TSS are shown with the arrows above the sequence. The position of Ets and Sp1 transcription factors binding is identical for both promoters, as shown by the light green, respectively, dark green, boxes on the sequence letters. The color scale at the left of the panels presents the probability for the formation of bubbles of amplitude  $>2.5$  Å with length  $L$  (vertical axis) beginning at a given nucleotide position (horizontal axis).

It was previously reported (17) that insertion of an Inr sequence in the promoter without changing the dispersed transcriptional window and transcription factors binding sites leads to the appearance of a new strong TSS at the Inr. The transcriptional activity of the wild-type sequence upstream of the insert was altered. The mutant TS exhibited more focused character, with the appearance of another pronounced TSS in addition to the Inr site.

The EPBD simulations of the TS-Inr mutant (Figure 3) revealed a significantly changed dynamic profile, compared with wtTS, with the appearance of a new and strong TSS located within the Inr insert, and a more ‘concentrated’ dynamic activity in the original wild-type sequence upstream of the insert (Figure 3). This dynamic pattern is in striking agreement with the reported (17) pattern of TSSs.

## DISCUSSION

The EPBD LMDs simulate DNA transient openings in the picosecond-time scale, a range that is to be expected from the strength of the hydrogen bond potential (1–20 meV) and is consistent with experimental studies of the vibrational frequencies of biomolecules (22–25). While the accuracy of the predicted sequence dependence

of DNA breathing frequencies has not been directly verified experimentally, the PBD simulations faithfully reproduce a variety of phenomena directly linked to the hydrogen bond stretching and unstacking (26–29). These include accurate prediction of unzipping force measurements (28), bubble nucleation size (29) and melting profile predictions (1,26,27). Given the striking accuracy of the melting temperature predictions of EPBD, including homopolymers and repeats that are difficult to model (1), we anticipate that the pre-melting dynamic trajectories produced by EPBD simulations are at least qualitatively correct.

The results reported here are consistent with our previous findings that core promoter TSSs exhibit specific breathing dynamic signatures (4). Further, the new data suggest that the observed transient collective openings in the DNA double helix are not simply a coincidental property of the TSS sequence, but actually play an important role in determining the TSS strength and position. Such collective openings may seed the formation of the transcriptional bubble needed for transcription, or they may represent another, yet unknown but important conformational feature of promoter DNA (3). In any event, the data rule out the possibility that the introduced mutations in the SCP1 promoter directly disrupt specific DNA–transcription factor contacts. These mutations were chosen to avoid points of contact with the pre-initiation complex, as identified in thorough DNase I footprinting studies of SCP1 and SCP1 mutants (16). Our gel shift experiments confirm that transcription factor–DNA interactions are indeed unperturbed.

The data indicate that the dynamics at the TSS are as important as the binding of the basal transcriptional factors for determining the transcription initiation strength, and it is thus a ‘localizer’ of the TSS position. Mutations in the MTE and DPE elements that prevent TFIID binding (12) result in decrease in SCP1 activity comparable ( $\sim 3$ - to 4-fold in luciferase reporter assay) with the impact of silencing the transcriptional bubble without perturbing TFIID binding (4-fold in Q-PCR assay). Curiously, a mutation inside the Inr motif of SCP1 was reported to suppress transcription more potentially (15-fold in luciferase reporter assay) (16). EPBD dynamic simulations revealed that, in addition to the reported inhibition of TFIID binding, this Inr mutant has significantly reduced dynamic activity (Figure 1C), consistent with our hypothesis that both DNA dynamics and transcription factor binding are determinants of transcription initiation.

The lack of sharply defined TSS-specific dynamics observed in the TS promoter could explain the lack of a ‘focused’ TSS in this gene. Moreover, implanting a segment with localized TSS-like dynamics resulted in the appearance of a new strong TSS coinciding with the location of the dynamic maximum. Surprisingly, changes in the dynamic profile of the original promoter sequence resulting from the insert also reflect the experimentally observed transcriptional activity of this sequence. Considering that the known transcription factor binding sites for the TS promoter were intact in the Inr-insert variant, the reported experimental differences between

the TS and the TS-Inr promoters can be readily explained from considerations of DNA dynamics. In contrast, according to a purely ‘transcription factor-centric’ view of transcription initiation, insertion of an Inr segment out of context in terms of transcription factor binding sites may introduce a new start site at the Inr but should not otherwise affect the original TSS distribution.

In conclusion, we propose that transcription factor binding and dynamic activity are both necessary for cellular gene transcription and are interdependent. The EPBD dynamic model appears to be uniquely capable of describing the sequence dependence of DNA dynamic features that are functionally relevant to transcription. In cells, the TSS-specific DNA breathing dynamics are likely to depend not only on DNA sequence, but also to be regulated by transcription factor binding, chromatin and DNA methylation.

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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