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Characterizing the Relationship Between DNA Bending and Transcription Elongation By T7 RNA Polymerase
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It is well recognized that transcriptional repression is mediated by the binding of a repressor protein to the DNA template. Some repressors, such as the lactose repressor (LacR), bind two sites on the DNA, forcing the DNA into a tight loop which includes the promoter. Unlike other repression mechanisms such as steric blocking, RNA polymerase (RNAP) is not physically prevented from binding to the promoter in these loops; in fact, it binds with greater affinity. Furthermore, it has been shown that LacR-induced repression depends directly on the loop size - as the loop becomes larger, repression decreases although LacR binds with higher affinity. This apparent contradiction can be resolved by considering the mechanical stress imparted on the DNA: in this case, we hypothesize that as loops increase in size, LacR repression decreases because the loop itself becomes more flexible. In order to elucidate the potential role of mechanical stress in transcriptional regulation, we have developed an assay capable of measuring transcription from DNA minicircles sustaining various levels of bending stress comparable to repressor loops. Using fluorescently labeled molecular beacons capable of hybridizing to a predefined portion of the transcript we have been able to measure the transcriptional elongation rate of bacteriophage T7 RNAP. We hypothesize that, in the absence of regulatory proteins, bending stresses are sufficient to repress transcription. Indeed, preliminary data confirm that tightly looped DNA can inhibit T7RNAP elongation, and we are currently expanding the analysis to minicircles with different degrees of bending to fully explore this relationship. Our study establishes for the first time that DNA bending is sufficient to repress transcription and necessitates the consideration of template mechanics in other transcription systems particularly those involving repressors known to significantly deform DNA tertiary structure.

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A New Model For Elongation Complex Stability in RNA Polymerase - the "topological Lock"
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What gives rise to the characteristic stability of an elongating (but not initiating) RNA polymerase and why do all RNA polymerases possess an 8-10 bp RNA:DNA hybrid? Conventional thinking posits that this length of duplex, together with protein-nucleic acid interactions, yields the required thermodynamic stability. We have proposed instead that the wrapping of 8-10 bp of RNA around the template DNA provides a topological locking of the RNA into the complex, preventing collapse of the DNA bubble. We have previously demonstrated that complexes dissociate primarily from ''forward translocated'' states, in which forward movement of the complex without incorporation of nucleotides leads to a shortening of the hybrid, but also to an unthreading of the lock, allowing dissociation.
In complexes halted at the end of a homopolymeric stretch of T in the template DNA, the complementary RNA can slip back a base, reexposing a templating T, and allowing the incorporation of an additional A into the RNA. This slippage process repeats to generate a very long poly(A) tail. Our results show that upon depletion or removal of ATP, the RNA slips diffusively such that both the 3′ and 5′ ends of RNA extend out of the protein. Consistent with predictions of the topological lock model (but not of the thermodynamic model), these complexes are *more* stable than conventionally halted elongation complexes. We have further prepared halted complexes with 4 or 0 (zero!) hybrid base pairs and these complexes are also exceptionally stable, arguing that topological locking of the RNA, rather than thermodynamic stability, prevents complex dissociation. This has implications for mechanisms of termination and explains why phage, bacterial, and eukaryotic RNA polymerases all contain an 8-10 base pair hybrid, despite the very different sizes of the proteins.

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RNA-Dependent RNA Pausing or Taking a Long Coffee Break
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RNA-enzyme interactions are at the heart of many fundamental biological processes such as transcription, translation, and RNA silencing. Determination of the thermodynamics and kinetics of these interactions is crucial for the understanding of cellular and viral biology.
We have applied single-molecule magnetic tweezers (MT) to study transcription kinetics of RNA-dependent RNA polymerase from Bacteriophage Φ6 (Φ6 RdRP). During transcription RdRP binds to the antisense strand of the double-stranded RNA genome and polymerases a new sense strand while displacing the old one. In vivo, this reaction is repeated many a times to generate a pool of sense strands that are translated by the host to generate viral proteins. In MT sense strand was suspended between glass surface and a paramagnetic bead and hybridized to its complementary antisense strand to form dsRNA. AsΦ6 RdRP progresses along the antisense strand it unwinds dsRNA releasing the sense strand in a single-stranded form. The rate of this release was measured with MTs and used to analyze the transcription kinetics.
During a single round of transcription, Φ6 RdRP switches between a moving state and a pause state. We could show that the overall rate (i.e. including the moving and pause states) depends on the applied force while the instantaneous rate (i.e. including only the moving state) reveals no force dependence. Likewise the pause frequency does not show any force dependence while the analysis of pause duration demonstrated that the exit from the pause state is slower at forces below 15 pN. We have analyzed the obtained results in the context of RNA-enzyme interaction as well as Φ6 RdRP's unwinding ability.

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Non-Cooperative Interactions Between Transcription Factors and Clustered DNA Binding Sites Enable Graded Transcriptional Responses To Environmental Inputs
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A paradigm in transcriptional regulation is that graded increases in transcription factor (TF) concentrations are translated into digital on/off transcriptional responses by cooperative TF binding to adjacent cognate sites. Such digital transcriptional responses underlie the definition of anatomical boundaries during development. Here we show that NF-kB, a key TF controlling inflammation and immunity, is conversely an analog transcriptional regulator relying on the non-cooperative usage of clustered homotypic sites. Contrary to the paradigm, we observed that increasing concentrations of NF-kB are translated into gradual increments in transcription of target genes. We provide a thermodynamic interpretation of the experimental observations by combining quantitative measurements and a minimal physical model of an NF-kB-dependent promoter. We demonstrate that NF-kB binds independently to adjacent sites to promote additive RNA-Pol II recruitment and graded transcriptional outputs. These findings reveal a novel paradigm in the usage of clustered TF binding sites, which may be extensively applied to the biological conditions in which the transcriptional output is proportional to the strength of an environmental input.

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The Energetic Basis of Abortive Cycling in Transcription
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Abortive initiation, the release of small RNA transcripts during synthesis of the first 8-10 bases of a transcript, has been well documented in most single and multi subunit RNA polymerases, and has been shown to occur in vivo. Structural studies have prompted the 'scrunched intermediate' mechanistic model (an elaboration of the earlier stressed intermediate model), which proposes that compaction of the upstream template DNA within the enzyme and/or expansion of the bubble during initiation leads to instability and the release of abortive RNAs.
T7 and E. coli RNA polymerases represent the most well-characterized transcription systems and despite having no structural or evolutionary similarities, share very similar fundamental mechanistic features. In the initially transcribing abortive phase of both systems, the bubble expands as the initial RNA:DNA hybrid grows and the hybrid pushes on components of the enzyme: both key features in the proposed scrunching mechanism.
In this work, we directly test predictions of the scrunching model. The introduction of nicks or gaps into the template ('scrunched') strand should reduce stress and therefore reduce abortive. Similarly, the introduction of extra bases in this region should increase the release of abortive RNAs or shift their profile to