361-Pos
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.

362-Pos
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.

363-Pos
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.

364-Pos
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.

365-Pos
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

shorter lengths. For all of these modifications, our results show no systematic change in the abortive amounts or profile.
A much simpler model argues that short RNA:DNA hybrids are intrinsically unstable simply because of their short lengths. In both the T7 and E. coli systems, we show that stabilizing the hybrid by initiating transcription using a pyrene-conjugated RNA dinucleotide eliminates abortive cycling altogether (the pyrene is known to add stabilizing stacking interactions). This result further argues against the scrunched intermediate model in that addition of the extra pyrene bulk should increase steric stress and so increase abortive cycling.

366-Pos
An Abortive Isomerization Branch in the Transcription Initiation Pathway At a σ^{54} Promoter As Revealed By Single Molecule Fluorescence Microscopy
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Regulated transcription initiation is a complex process that involves multiple protein factors and a series of polymerase-DNA complexes that are intermediates in the reaction. This complexity presents a significant challenge for ensemble experiments that aim to elucidate the reaction pathway. We here report the kinetic mechanism of initiation at the σ^{54} promoter of the glnALG operon in *Salmonella typhimurium*. This prototypical activator-dependent promoter is regulated by nitrogen stress. To circumvent the complexity of ensemble analysis, we used multi-wavelength single-molecule fluorescence colocalization methods to follow initiation reactions on individual surface-anchored DNA molecules that contain σ^{54} promoters. Three distinguishable dye labels enabled us to follow reactions in which RNA polymerase binding, open complex formation, escape into transcription elongation and departure of the σ^{54} subunit were detected in individual transcription complexes, and the interconversion kinetics for all states were measured. Transcription initiation from this promoter occurs only following a polymerase isomerization that is induced by interaction with the NtrC activator protein in the presence of ATP. However, we observed that with NtrC present the polymerase leaves the promoter faster than the combined rates of initiation plus closed complex departure. Thus, a fraction of activator-mediated polymerase isomerizations displace the polymerase from the promoter without initiating transcript synthesis. This activator-induced abortive isomerization is a non-productive branch off of the initiation pathway and is more common than productive transcription initiation. We speculate that abortive isomerization is a consequence of the large energy input required to disrupt promoter-polymerase interactions prior to promoter escape. Taken together, our results define the full pathway and dynamics of initiation at this activator-dependent promoter and illustrate the power of multi-wavelength single-molecule colocalization methods in the elucidation of complex biological regulatory mechanisms.

367-Pos
Highly Bent DNA: A Novel Repressor of T7 RNA Polymerase
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The use of DNA templates sustaining varying degrees of supercoiling has established that mechanically stressed DNA can influence transcription by RNA polymerase (RNAP). However, the interpretation of supercoiling studies is complicated by the lack of a detailed description of the bending and torsional conditions present on length scales that are relevant to RNAP activity. A quantitative understanding of how bending and twisting DNA influence transcription has yet to emerge, largely owing to the lack of an assay capable of quantifying the transcriptional competency of an RNAP from DNA templates sustaining well-defined levels of mechanical stress in the absence of supercoiling or other DNA-binding proteins. To directly test the hypothesis that mechanical stress imparted to tightly looped DNA is sufficient to repress transcription, we have developed an assay capable of quantifying the ability of bacteriophage T7-RNAP to transcribe circular transcription templates on the order of 100bp in size, thus restricting our observations only to the effects of mechanical stress on transcription. By encoding the promoter sequence for T7-RNAP within minicircles 100bp, 106bp, and 108bp in size, we have also characterized the effects of three distinct torsional stress states (within comparably bent minicircles) on the transcriptional activity of T7-RNAP. From these minicircle templates, we observe that the elongation velocity and processivity of T7-RNAP is reduced by roughly two orders of magnitude, confirming that highly bent DNA alone is capable of repressing transcription. Additionally, we observe a fivefold enhancement of elongation velocity as the template is untwisted, a finding qualitatively supported by previously reported observations. Our results establish that DNA mechanics can directly control RNAP activity, and given the required use of DNA templates by all RNAPs, necessitate the consideration of template-mediated effects in repression studies.

368-Pos
Single Molecule Study of Promoter Search By E Coli RNAP
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During transcription initiation, RNA polymerase (RNAP) must find specific promoters in the genome in response to different physiological conditions. It has been suggested that 1-D sliding along DNA may accelerate this process so that it can be faster than 3-D diffusion limit. However contradicting ensemble and single molecule experiments have reported drastically different 1-D diffusion coefficients ($10^{1}\mu m^2 s^{1}$ vs. 10^{2} and $10^{5}\mu m^2 s^{1}$). Here we used our high throughput single molecule technology to simultaneously observe hundreds of double tethered lambda DNA molecules in an effort to determine how Qdot-labeled *E. coli* RNAP searches for promoters. Using this system we have observed specific binding to known promoters, formation of heparin resistant open complexes, and transcription from know promoter regions. Analysis of the time courses of promoter search showed two populations: The first population binds DNA nonspecifically and dissociates with an average life time 3.5 sec; The second population binds DNA specifically to the promoter regions and never comes off within our observation time. We have not observed evidence of extensive 1D diffusion with either population, and we estimate upper boundaries for the diffusion coefficients and sliding lengths of $10^{4}\mu m^2 s^{1}$ and 170bp, respectively; these values are much smaller than reported by ensemble experiments. Our data suggest that 3-D diffusion is the main pathway for *E. coli* RNAP to search for promoters and 1-D sliding does not play a significant role in this process. The biological context of this result is discussed.

369-Pos
Structural Modeling of PhoB Dimer and Its Interaction With RNAP Complex
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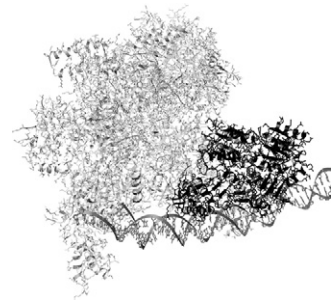
PhoB is a response regulator of the two-component signal transduction system. Structurally, PhoB can be divided into two domains. A N-terminal Receiver Domain (ED) that adopts a flavodoxin-like fold shared by receiver domains of other response regulators. The C-terminal Effector Domain (ED) of PhoB adopts a winged-helices fold that recognizes and binds to its targeted DNA duplex. Structures of PhoB molecule have been well-studied including the homodimers of the ED (PDB accession code: 1GXP), the RD (PDB accession code: 2JB9) and the two domains structure (PDB accession code: 1KGS). However, the functional form (DNA-binding) of the PhoB two-domains structure is still not available. Here, we engaged in an exercise to develop a structural model of the molecule in its dimeric functional form binding to its targeted DNA duplex. The model was developed using the observed crystal contacts between the domains of various response regulators. The modeled structure of PhoB/DNA complex is assembled into the RNAP/DNA complex (also modeled by our group) to study the interactions between PhoB and RNAP as shown in the attached figure.



370-Pos
Quantitative Studies of Transcription in E.coli With Subdiffraction Fluorescence Microscopy
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The organization of biomolecules into macromolecular assemblies is often closely related to biomolecular function. However, such structures often remain unresolved using conventional light microscopy. By applying novel high-resolution single-molecule fluorescence techniques, it becomes possible to study biomolecular structure and interaction below the diffraction limit of light, reaching a lateral resolution of ~20 nm [1, 2]. We use photoswitchable and photoactivatable fluorescent probes in combination with direct stochastic optical reconstruction microscopy (dSTORM) [2] and photoactivation-localization microscopy (PALM) [3]. Following light-induced activation of a subset of fluorescent probes attached to target proteins, the fluorescent state is read out and single emitters are localized with nanometer precision. This procedure is