IMI•DNA from the absolute minimum. An analysis of various contributions to the PMF reveals that solvent effects play an important role for the largest and more flexible drug DSI. Instead, the PMF of IMI•DNA overall correlates with changes in the binding enthalpy. Implications of these results on the sequence-selectivity of the two drugs are discussed.

255-Pos Competitive Exchange Equilibria Under Mechanical Forces: A New Paradigm For Biosensors

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Board B88

We are developing a new class of biosensors that, contrary to existing methods, relies on the breaking of pre-existing biomolecule complexes such as dsDNA or antibody-antigen. In these biosensors, micron-scale superparamagnetic beads are bound to the surface via one or multiple biomolecular tethers consisting of oligonucleotides bound to the surface of the device hybridized with oligonucleotides bound to micro-beads. When oligonucleotides complementary to those on the surface are injected into the system, competitive binding ensues in which the free (analyte) oligonucleotides can replace the bead oligonucleotides in hybridizing with the surface oligonucleotides, breaking the tethers and releasing the beads. The latter functions as the signal transduction. A similar system employing antibody- antigen tethering of the micro-beads can also be envisioned. In the case of nucleic acid tethers, the equilibrium between the analyte molecules and the bead molecules binding with the surface molecules can be changed by controlling the length of the complementary region of both the analyte and micro-bead oligonucleotides. Alternatively, a magnetic force stretching the tethers can be relied on to shift the equilibrium in favor of breaking the tethers. To uniformly apply forces, a magnetic tweezers setup is used. Force/unbinding curves were obtained to characterize the tethers when the number of tethers per bead was varied from one to hundreds per bead. Analyte oligonucleotide injected at low force increased the rate of micro-bead detachment compared to blank buffer injections. The sensitivity of the device scales with the number of tethers attaching individual micro-beads.

256-Pos What Configurational Diffusion Coefficient Characterizes Loop Formation In ss-polynucleotides?

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Board B89

Numerous kinetics measurements on the formation of single-stranded (ss) DNA and RNA hairpin structures with ~4–20 nucleotides (nt) in the loop and ~5–8 base-pairs in the stem, indicate that the time required to form hairpins is ~10–500 microseconds. If ss-polynucleotide chain is treated as an ideal semiflexible polymer with a statistical segment length of ~4 nt, the theoretical estimate for the end-to-end contact time for an ~10-nt long chain is expected to be tens-of-nanoseconds. To explain this discrepancy in time-scale, we proposed that the formation of the nucleating loop, prior to the zipping step, is slowed down as a result of transient trapping in misfolded conformations, with mis-paired base-pairs, non-native hydrogen bonding, or intrastrand stacking interactions in the unfolded state. Experimental measurements of end-to-end contact formation indicate that loop closure times for 4-nt poly(dT) loops are ~400 nanoseconds, and for 4-nt poly(dA) loops are ~8 microseconds, thus confirming that intrachain interactions slow down the configurational diffusion of the chain (Wang and Nau, *J. Am. Chem. Soc*. 2004, ***126***, 808). Interestingly, despite this evidence for intrachain interactions slowing down diffusion, the hairpin closing times for both ssDNA and RNA hairpins are found to scale with the length of the loop as $L^{2.2–2.6}$, in reasonable agreement with the scaling behavior expected for loop-closure of a semiflexible polymer.

Here, we present a kinetic zipper model that explicitly includes all misfolded microstates with non-native contacts, to describe the hairpin closing times. The loop-size dependence is described within the framework of a wormlike chain model, to obtain a characteristic configurational diffusion coefficient that is relevant for formation of the nucleating loop.

Transcription

257-Pos Byophysical Modeling of Transcription Initiation by Bacterial RNA Polymerase

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Board B90

The mechanism by which RNA polymerase (RNAP) forms the open complex (the first step in transcription initiation) is still unknown, despite two decades of intensive experimental research. To distinguish between several qualitative hypotheses, we develop the first quantitative model of the open complex formation by bacterial RNAP [1]. We derive an explicit relationship that connects transcription initiation rate with physical properties of DNA sequence and DNA-RNAP interactions. We compare our model with both biochemical measurements and genomics data and report a very good agreement with the experiments, with no free parameters used in model testing. The agreement strongly supports both the quantitative model that we propose and the qualitative hypothesis on which the model is based. Bioinformatic applications of our model, which allow efficient analysis of kinetic properties of DNA sequences on the whole genome scale [2], will also be addressed.

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258-Pos Single-molecule Kinetic Studies Of Transcription Initiation

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Board B91

Recent single-molecule work showed that initial transcription and abortive initiation (the iterative process of synthesis and release of short RNAs before promoter escape) both occur through a DNA-scrunching mechanism. The kinetics of abortive initiation have also been studied using total internal reflection fluorescence (TIRF) microscopy on surface-immobilized transcription complexes (Margeat et al., *Biophys J*, 2006, *90*, 1419–31); however, the limited temporal resolution of the earlier study (400 ms per frame) did not allow observations of conformational changes at the single-basepair step during the translocation of RNA polymerase (RNAP) on DNA. Moreover, no real-time observation of initial transcription and promoter escape has been performed using FRET.

To address these limitations, we devised conditions that improved our temporal resolution for observing transcription complexes by an order of magnitude (~30 ms per frame). This involved building a TIRF microscope with improved sensitivity; use of brighter fluorophores; and improved buffer additives that limit photobleaching and reduce fluorophore photophysics.

To study the kinetics of abortive initiation at the *lac* promoter, we studied $RP_{itc<=7}$ (an initial transcription complex synthesizing RNA of up to 7 nucleotides in length). The complex was prepared by immobilizing biotinylated RNAP-DNA open complexes on a modified glass slide (coated with biotinylated-polyethyleneglycol and streptavidin) and adding ApA, UTP and GTP. Our results show FRET fluctuations between low and high FRET states on single $RP_{itc<=7}$ complexes; the fluctuations most likely represent conformational changes due to DNA scrunching, with each fluctuation corresponding to synthesis of single RNA strand. The timescale of fluctuations is similar to that of abortive RNA synthesis. It was also observed that the return to the initial low-FRET state due to RNAP backward translocation occurs in multiple steps. Analysis of intermediate states as well as the dynamics in the open complex is currently in progress.

259-Pos Single-Molecule FRET Studies Of DNA Dynamics In Transcription Initiation

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Board B92

Gene transcription involves multi-step transitions that result in complexes at different conformational states; in earlier work, we studied such states using single-molecule FRET. To improve our capability to resolve subpopulations of complexes and monitor their dynamics, we developed strategies to increase the photon count rates per molecule and decrease the statistical noise of FRET measurements. These strategies sharpened the distribution functions of populations, resolving closely spaced distributions and narrowing the width of distributions (a parameter which can report on molecular dynamics).

To monitor dynamics in the RNAP-promoter DNA open complex (RP_o), we used doubly labeled *lac* promoter DNA fragments to form open complexes and study them using single-molecule FRET. Our results show that, surprisingly, upon forming the open complex, the width of FRET efficiency distributions increase substantially compared to free DNA, becoming much larger than the width predicted on purely statistical terms ("shot noise"). Upon addition of ATP (which leads to synthesis of dinucleotide pppApA), distributions become narrower; widths narrow further upon adding initiating dinucleotide ApA.

Our results suggest that RPo is highly dynamic and that DNA has substantial lateral mobility (a feature also exploited during DNA scrunching in initial transcription). In RPo, DNA is anchored at the -10 element, just upstream of the transcription bubble; however, the downstream DNA can explore transcription start sites with variable spacing from the -10 element, with the most probable state at or adjacent to the utilized transcription start site. Upon addition of ApA, the base-pairing of the dinucleotide with the template "anchors" the DNA to the rest of the complex, reducing DNA mobility and FRET-distribution widths. Our results are important for understanding utilization of alternative transcription start sites and transcriptional regulation by initiating nucleotides; related studies are in progress.

260-Pos Interactions Between RNA Polymerase And Initiation Factor τ

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Board B93

To carry out sequence-specific transcription initiation, bacterial RNA polymerase (RNAP) must associate with the initiation factor σ. σ contains five principle conserved regions: σR1.1, σR2, σR3, the σR3/σR4 linker, and σR4. σR2, σR3, and σR4 are structured domains that contain determinants for sequence-specific interactions with promoter DNA. σR1.1 and the σR3/σR4 linker are unstructured, highly negatively charged, segments.

In this work, using fluorescence resonance energy transfer assays, we have defined energetics and kinetics of interactions between *E. coli* RNAP and *E. coli* σ^{70}, and we have defined contributions of individual conserved regions of σ^{70}.

We find that the free energy of RNAP-σ^{70} interaction is -15.6 kcal/mol under standard transcription conditions. We find that σR1.1, σR2, σR3, the σR3/σR4 linker, and σR4 all contribute to the free energy of RNAP-σ^{70} interaction under standard transcription conditions (apparent contributions of 2.8, 9.7, 0.2, 1.1, and 1.8 kcal/mol, respectively). The free-energy contribution of σR1.1 is strikingly salt-dependent (2.8 kcal/mol at 0.1 M NaCl; 0.5 kcal/mol at 0.25 M NaCl). We infer that the free-energy contribution of σR1.1 is electrostatically dominated, and we suggest that salt-dependence

of the free-energy contribution of σR1.1 may mediate switching between σ^{70} and alternative σ factors under high-salt stress in vivo.

We find that the association rate constant for RNAP-σ^{70} interaction is $2.3 \times 10^8\ M^{-1}s^{-1}$, which is close to the diffusion-controlled limit. We find that deletion of σR1.1, σR3, the σR3/σR4 linker, and σR4 does not affect the association rate constant for RNAP-σ^{70} interaction. We infer that only σR2 is important for initial RNAP-σ^{70} interaction, and that RNAP-σ^{70} interaction proceeds through a multistep mechanism, involving initial interactions by σR2 followed by interactions by other σ conserved regions.

261-Pos Formation of RNAP-promoter Open Complex: Kinetic Site-specific Protein-DNA Photocrosslinking

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Board B94

We have used quench-flow rapid mixing together with pulsed-laser flash photolysis to define the pathway and kinetics of interactions between RNA polymerase holoenzyme (RNAP) and each of two promoters: a consensus promoter (*lacCONS)* and a non-consensus promoter (*lacUV5*). For each promoter, we performed kinetic RNAP-DNA photocrosslinking using promoter derivatives containing phenyl-azide photoactivatible crosslinking agents incorporated at a single, defined sites. Probe sites were chosen to report RNAP-DNA interactions in key segments of the promoter: i.e., the −42, −35, −20, −10, +1, and +10 regions.

The results for the consensus promoter define a single intermediate in formation of RNAP-promoter open complex (RP_o) at the consensus promoter:

RP_c (partial interactions in −42, −35, −20, and −10 regions)
RP_o (full interactions in −42, −35, −20, −10, +1, and +10 regions)

The results define three intermediates in formation of RP_o at the non-consensus promoter:

RP_{c1} (partial interactions in −35 and −10 regions)
RP_{c2} (partial interactions in −35 region; full interactions in −10 region)
RP_{c3} (partial interactions in −35 region; full interactions in −20, −10, and +10 regions)
RP_o (full interactions in −42, −35, −20, −10, +1, and +10 regions)

Our results establish that formation of RP_o does not involve a unique pathway. Both the number of intermediates and the identities of intermediates differ at different promoters.

Our results further establish that RNAP makes two successive, qualitatively different, sets of interactions with the −35 and −10 regions in formation of RP_o: a "preliminary" set of interactions and a "final" set of interactions. At the promoters studied, the transition between preliminary and final sets of interactions accompanies formation of RP_o in the case of the −35 region and accompanies or precedes formation of RP_o in the case of the −10 region.

262-Pos Efficiency And Versatility Of Distal Multisite Transcription Regulation

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Board B95

Gene expression is typically controlled by the assembly of macromolecular structures on multiple distal DNA sites that are brought close to each other by the formation of DNA loops. This molecular complexity is present even in the simplest genetic systems, such as the prototypical lac operon. We have implemented a statistical thermodynamics approach that naturally incorporates the effects of multiple DNA loops between different DNA sites into gene regulation models. Our approach applied to the lac operon, besides accurately predicting the cellular behavior for many environmental conditions and genetic backgrounds, reveals that the presence of at least three DNA binding sites with looping provides a mechanism to combine robust repression with sensitive induction, two seemingly mutually exclusive properties that are required for optimal functioning of metabolic switches.

263-Pos Single-Molecule Fluorescence Studies of T7 RNA Polymerase (T7 RNAP) Transcription

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Board B96

Bacteriophage T7 RNA polymerase (RNAP) is a single subunit RNA polymerase that is well characterised structurally and functionally. However, there are still major conformational changes during T7 RNAP transcription that remain unclear: for example, X-ray crystallographic studies showed large-scale reconfiguration of several protein modules during the transition from initiation to elongation. However, the sequence, kinetics, and determinants of such conformational changes have not been determined. Single-molecule fluorescence studies of T7 RNAP will help characterise such conformational changes.

Here, we describe single-molecule FRET studies of T7 RNAP elongation complexes. Site-specifically labelled T7 RNAP and DNA fragments were used to monitor translocation of T7 RNAP along DNA using a leading-edge FRET assay (as in Kapanidis et al., Mol Cell 2005, *20*:347). Experiments with diffusing complexes show stable stalled elongation complexes with low FRET efficiency, consistent with the location of the probes and the crystal structure of the elongation complex. Upon resuming transcription, we observe T7 RNAP downstream translocation as a FRET increase; addition of

all nucleotides "chases" ~100% of active T7 RNAP molecules from the DNA, demonstrating the high activity of elongation complexes. We extended this work by using Total Internal Reflection Fluorescence (TIRF) microscopy and dual-view imaging to study immobilised elongation complexes; our results were consistent with the measurements on diffusing complexes. Work is in progress to achieve single base-pair translocation resolution in real-time and to address the nature of promoter-escape–related conformational changes raised by the X-ray structural models.

264-Pos GreB Residence Time On RNA Polymerase And Elongation Complexes

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Board B97

The *E.coli* transcription factor GreB binds to RNA polymerase and modulates processes in both initiation (e.g., accelerating promoter clearance) and elongation (e.g., rescuing complexes that are backtracked due to arrest or misincorporation). To perform these functions, GreB must bind to a variety of different transcription complex configurations, but the relative strengths and longevities of these various binding interactions have not been characterized. We used fluorescence correlation spectroscopy of Cy3-labeled GreB in the presence of RNA polymerase or elongation complexes to measure stabilities and lifetimes of the binding interactions. The residence time of GreB on either core polymerase or sigma70 holoenzyme is extraordinarily long, with mean durations 54 ± 3 s and 30 ± 4 s, respectively. These values are compatible with the previously reported tight equilibrium binding and raise the possibility that some holoenzyme molecules arrive at promoters with GreB already bound. In contrast, GreB association with functional reconstituted elongation complexes is too weak (dissociation equilibrium constant $>$ ~100 nM) and/or too kinetically unstable (residence time $<$ 1 s) to be detected in these experiments. Even though GreB interacts only weakly with generic transcription complexes, it remains possible that the protein binds more stably, and is therefore preferentially recruited to, backtracked transcription elongation complexes. We prepared reconstituted elongation complexes with non-complementary 3′ RNA ends that are stably backtracked by either 2 or 6 nucleotides. The RNA backbone phosphates following each of the final three complementary bases were substituted with phosphorothioate groups, greatly reducing the rate of RNA cleavage. Using single-molecule total internal reflection fluorescence microscopy to look at GreB specific elongation of backtracked complexes, we are currently investigating whether GreB has a longer residence on these stably backtracked complexes than on non-backtracked complexes.

265-Pos Single-molecule Study Of The Effects Of NusG On Transcriptional Pausing

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Board B98

Transcriptional pausing plays an essential role in the regulation of gene expression and is modified by timely interactions with a number of regulatory factors. In the prokaryotes, NusG is a universally conserved accessory protein that modulates sequence-dependent pausing and is essential to cell viability. Although NusG is known to increase the average rate of elongation, it is not known whether it accomplishes this by increasing the active elongation velocity between pauses or by decreasing the efficiency and/or lifetime of transcriptional pauses. We studied the effects of NusG on transcription under varying loads and NTP concentrations by employing a dumbbell optical-trapping assay with high spatiotemporal resolution to follow the motion of individual molecules of RNA polymerase transcribing a DNA template. From individual transcription records, we were able to determine the pause-free elongation rate, as well as the frequency and duration of long and short-lifetime pauses. We also performed parallel experiments in the presence of inosine triphosphate, which is known to increase the frequency of long pauses related to nucleotide misincorporation. We found that the presence of NusG only exerts small effects on the pause-free velocity and the properties of short-lifetime pauses, but has a significant effect on the frequency of long-lived pauses.

266-Pos Single-molecule Fluorescence Studies Of sigma54-dependent Transcription

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Board B99

In bacteria, a multi-subunit RNA polymerase (RNAP) directs transcription after forming a functional complex ("holoenzyme") with transcription-initiation proteins known as sigma factors. Most of the published work focuses on sigma70-dependent transcription; here, we study sigma54-dependent transcription, a functionally distinct mode of bacterial transcription which has similarities with eukaryotic transcription (both require ATP hydrolysis, enhancer-DNA sequences, and activator proteins). Here, we present studies of

sigma54-dependent transcription using single-molecule fluorescence resonance energy transfer (smFRET) and gel electrophoresis. To study the mechanism of abortive initiation (a mode of transcription during which RNAP synthesizes and releases short RNA fragments, <10 nucleotides in length) in sigma54-dependent transcription, we monitored distance changes between different parts of the transcription complex during abortive initiation. Our results show that upon synthesis of RNA of up to 5 nucleotides in length, there is relative movement of the DNA downstream of the transcription bubble vs. the DNA upstream of the transcription bubble. This movement is eliminated by the addition of rifampicin, an antibiotic that blocks initial transcription. We are currently measuring other distances within the transcription complex to identify which of its parts move during abortive initiation.

Furthermore, we are studying sigma54-dependent DNA opening by monitoring the FRET efficiency between two complementary fluorophores placed on opposite strands of the transcription bubble. Parallel experiments using sigma70-containing transcription complexes confirm the feasibility of this single-molecule DNA-opening assay, which also reports on the process of promoter escape and DNA reclosing.

267-Pos Controlling DNA Condensation in Artificial Systems

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Board B100

In the cell nucleus, DNA is organized by binding to the positive surface of histone proteins, wrapping twice around each histone and folding itself into a large superhelix known as chromatin. The chromatin is more or less condensed depending on the local environment, and genes in readily condensed chromatin display little or no transcriptional activity. We are developing methods for artificial DNA condensation in order to mimic such functions in cell nuclei and thereby control transcription *in vitro*. Our strategies involve polycations with tunable charge, as well as polyamidoamine (PAMAM) dendrimers which are thought to function as synthetic "histones" by providing a large cationic surface with well defined charge density. The nature of the dendrimer surface may easily be modified. The objective of our research is to develop synthetic simple analogues of cell nuclei that may be triggered to allow or prevent transcription of an artificial genome. These nuclei analogues, either immobilized on a surface or implanted into cells, will provide novel strategies in industrial production of proteins and other complex biomolecules.

Using an *in vitro* transcription-translation assay, we have found that complexing DNA with PAMAM dendrimers results in reduced, but not inhibited, transcriptional activity. Through ultracentrifugation experiments, we are able to show that dendrimers bind to DNA in a highly cooperative manner, which explains how some DNA can still be available to the transcription machinery. The DNA fraction where transcription is inhibited can easily be purified. The amount of DNA condensation is investigated using polarized-light spectroscopic techniques, and fluorescence-based methods are used to monitor the availability of condensed DNA to ligands. Some approaches to resolve the higher-order structure of the condensed DNA will also be reported.

268-Pos Analyzing the Effects of mRNA Processing Forces on Transcription Rates In Vivo

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Board B101

The RNA polymerase enzyme is regarded a potent molecular engine. *In vitro* studies with single molecules of *E.coli* RNAP have shown that the enzyme can work efficiently in spite of strong applied forces. We are examining how transcription rates are controlled *in vivo* within the mammalian nucleoplasmic environment encountered by the polymerase. In the cell nucleus, co-transcriptional processes such as mRNA splicing are taking place as mRNA transcription proceeds. These dynamic processing events include the deposition and release of extremely large molecular complexes, and are occurring simultaneously as Pol II moves through a tangle of chromatin and other nucleoplasmic constituents. It is unknown whether the working rates of the RNA polymerase are affected by such processes and forces. It is now possible to follow a specific gene[1] and its mRNA *in vivo*[2]. Using a fluorescence microscopy approach we have quantified Pol II transcription rates *in vivo* and have identified the kinetics of polymerase pausing[3]. We now ask whether mRNA processing events that are occurring co-transcriptionally, can affect the kinetics of the polymerase at the site of transcription. We generated stable cell lines with integrated inducible genes that contain increasing numbers of introns/exons, or that are intronless. We identify active transcription sites in single living cells, and using FRAP or photoactivation techniques we measure the *in vivo* rates of mRNA synthesis on these different genes. Our findings demonstrate the potency of polymerase processivity *in vivo,* and show that the frequency of promoter firing rather than downstream processing events influence transcription rates.

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269-Pos Applied Force Reveals the Mechanism and Energetics of Transcription Termination

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Board B102

In prokaryotes, transcription termination is generally triggered by specific DNA sequence elements that encode an RNA transcript consisting of a GC-rich hairpin followed by a 9 nt U-rich tract. Although these sequence elements have been identified and characterized biochemically, questions still remain about the physical mechanism by which they lead to destabilization of the transcription

elongation complex (TEC). The forward translocation model proposes that formation of the terminator hairpin pulls on the 5′ end of the RNA, driving the RNAP molecule downstream along the DNA template without transcript elongation and shortening the RNA:DNA hybrid by up to several base pairs. The U-rich tract, which is also necessary for termination, is thought to facilitate transcript release by forming a comparatively weak RNA:DNA hybrid in the RNAP active-site cleft. To investigate the mechanistic role of these sequence elements, we used single-molecule optical trapping techniques to exert controlled forces on either the DNA template or the RNA transcript of individual E. coli TECs as these transcribed three representative terminator sequences (his, t500, and lambda tR2). Assisting or hindering force exerted through the DNA did not affect termination efficiency (TE) in WT terminators, indicating that the commitment step in termination does not likely involve forward translocation of RNAP along the DNA. However force-dependent transcript-release kinetics of the t500 terminator and the TE force-dependence of a t500 mutant suggest a forward translocation mechanism for this particular terminator. Tension along the RNA at terminator U-tracts (without hairpins) caused force-dependent transcript release, likely through shearing of the RNA:DNA hybrid. We deduce that formation of the last terminator hairpin bases supplies energy to destabilize the hybrid, and propose a quantitative energetic model that predicts the force-dependent TE of three WT terminators and the bulk TE of mutant terminators.

270-Pos Direct Spectroscopic Study of Reconstituted Transcription Complexes Reveals that Intrinsic Termination is Driven Primarily by Thermodynamic Destabilization of the Nucleic Acid Framework

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Board B103

We have used near UV circular dichroism (CD) and fluorescence spectra of site-specifically placed pairs of 2-aminopurine (2-AP) residues to probe the roles of the RNA hairpin and the RNA-DNA hybrid, separately and together, in controlling intrinsic termination in transcription complexes containing T7 RNA polymerase (RNAP). Nucleic acid scaffolds were formed by direct mixing of defined sequences of DNA and RNA oligonucleotides to form non-promoter initiated transcription complexes. The scaffolds containing RNA hairpins immediately upstream of a GC-rich hybrid formed complexes of reduced stability, while the same hairpins adjacent to a hybrid consisting of rU-dA base pairs resulted in complex dissociation. 2-AP probes located at the upstream ends of the hairpin stems show that the hairpins open on RNAP binding and that stem re-formation begins after one or two RNA bases on the downstream side of the stem emerge from the exit tunnel of the RNAP. We show that hairpins directly adjacent to the RNA-DNA hybrid weaken RNAP binding, decrease elongation efficiency, and disrupt the upstream end of the hybrid, as well as interfering with the movement of the template base at the RNAP active site. Probing the upstream edge of the DNA transcription bubble demonstrates that such hairpins prevent upstream translocation of the RNAP on complex formation, suggesting that the hairpins transiently 'lock' the polymerase to the nucleic acid scaffold and thus hold the RNA-DNA hybrid 'in frame'. At intrinsic terminators the weak rU-dA hybrid and the adjacent termination hairpin combine to destabilize the elongation complex sufficiently to permit significant transcript release, while hairpin-dependent pausing provides the time required for the process to go to completion.

271-Pos Shades of Gray in the Binary On/Off Lac Repressor Paradigm

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Board B104

The control of the *E. coli lac* operon via its inducible repressor/activator complex competition has become the paradigm of both prokaryotic and eukaryotic transcriptional regulation at its most basic level. But our understanding of this fundamental system remains incomplete, especially with regards to the action of the Lac repressor, which does not result in simple, binary on/off transcriptional states but rather in degrees of repression depending on whether or not the repressor tetramer is allowed to simultaneously bind two of the multiple operator sites present in the *lac* operon, thereby looping the operon's DNA, and what the probability of forming these loops is (1,2). Recent work in our lab using the single-molecule technique of tethered particle motion (TPM) has demonstrated strong effects of loop length, loop flexibility, and repressor concentration on this looping probability (3). However, our work and the work of other labs has so far focused on observing looping between two operator sites, whereas in wild-type cells the operon contains three operators: O_1, the strongest, which overlaps the promoter; O_2, slightly weaker and located 401 bp downstream in the coding region of the *lacZ* gene; and O_3, the weakest, located 92 bp upstream of O_1. To explore the effect of having three, rather than just two, operators present, we have used the TPM assay to examine looping by the wild-type operon DNA as a function of Lac repressor concentration.

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272-Pos Counting mRNA Copy Numbers In Single *E. Coli* Cells Reveals Non-Poissonian mRNA Distributions

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Board B105

Detecting mRNA in a single cell is essential to understanding cell-to-cell variations (noise) in gene expression of a genetically identical population and transcription mechanisms. Since most bacterial genes express low copy number of mRNAs, single molecule detection sensitivity is required to measure the mRNA copy numbers. Here we use "digital PCR" to count specific mRNA molecules at low copy numbers in individual *E. coli* cells. The high sensitivity of real-time PCR allows detection of a single copy of mRNA molecule within a single-cell. By counting genomic DNA together with mRNA, we removed the heterogeneity of mRNA copy number due to the variation in gene copy numbers. This allowed us to map the distribution of mRNA molecules expressed from a constitutive promoter (P_{A1}) and *lac* promoter (P_{lac}) among a cell population. In contrast to the Poissonian distribution observed under highly repressed conditions, we consistently observed non-Poissonian mRNA distributions under the fully induced conditions. To shed light on the underlying transcription mechanism, we compared the data with different models for transcription.

DNA Replication, Recombination & Repair

272.01-Pos A Computational Study Of The Non-covalent Interactions Between The K-ras Gene And Some (BPDE And Acrolein) Carcinogens

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Board B105.01

The K-ras gene is intimately related to cancer as it modulates various intracellular signal pathways that control, among other things, cell growth. Some types of cancer are linked to mutations in the K-ras gene. In some human cancers, codons 12, 13 and 61 of the K-ras gene are frequently mutated. Benzo[α]pyrene, a polycyclic aromatic hydrocarbon, and acrolein, an aldehyde, are two known carcinogens. Benzo[α]pyrene is found in cigarette smoke and automobile exhaust fumes and it needs to be metabolized before it can react with DNA. (+)-anti benzo[α]pyrene diol epoxide is the ultimate carcinogenic form of benzo[α]pyrene. Acrolein can also be found in cigarette smoke and, being a most abundant, reactive and mutagenic aldehyde, needs not be activated to damage DNA. We have minimized free energy functions with the aid of genetic algorithms to compute the non-covalent interactions between the exon 1 of the K-ras gene and the carcinogens BPDE and acrolein. For both carcinogens, we found important potential binding (docking) sites for which the Van der Waals interaction is dominant. Some of these docking sites are related to potential DNA damage after the covalent bondings have taken place.

272.02-Pos The Role of γ-phosphate Binding in the Catalytic Mechanism of dUTPase

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dUTPase is the unique enzyme that catalyses the pyrophosphorolysis of dUTP, thus regulating the extent of uracil incorporation into DNA. Massive uracil incorporation into DNA leads to cell death, dUTPase has therefore been recognized as a high-potential drug target in cancer. The atomic structure of several different dUTPase isoforms is known; however, detailed solution studies on the mechanism of eukaryotic dUTPases were completely missing and thus we endeavored to fill this gap.

The present work focuses on the catalytic mechanism of human dUTPase, and is a continuation of our recently published study which revealed the fundamental steps of the enzymatic cycle and provided a quantitative model for the mechanism. Our specific interest now is in deciphering the structural-functional reasons that lead to the hydrolysis of the α-β-phosphate linkage only and specifically in the presence of the γ-phosphate. We seek to understand why dUDP is not hydrolyzed by dUTPase despite the presence and similar coordination of the α-β-phosphates in dUDP and dUTP. To address the above issues, several transient kinetics and equilibrium enzymological as well as spectroscopic methods were employed using active-site mutant human dUTPase enzymes. We engineered active-site mutants specifically to disrupt interactions between the protein and the γ-phosphate moiety of the nucleotide. We found that perturbation of the p-loop-like γphosphate binding-site of dUTPase resulted in a similar K_d for dUTP and dUDP and that it prevented an isomerization event at the active-site. Enzymatic activity is reduced to various extents depending on the specific mutation and correlates with the residual ability of the protein to bind the γphosphate moiety. Based on our observations we now hypothesize that binding of the γ-phosphate causes a relatively slow isomerization of the active site, which develops strain within the nucleotide phosphate-chain aiding catalysis.

272.03-Pos MutM Interrogating Normal Purine Bases in Its Active Site

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MutM is a bacterial DNA glycosylase that specifically recognizes oxidatively damaged DNA bases and initiates the base excision