processes far from thermodynamic equilibrium. Therefore, the intracellular dynamics are complex and subject to a multitude of constraints and forces. We study the conformational fluctuations of chromosomal DNA in vivo in live and dead *Escherichia Coli* cells by Fluorescence Correlation Spectroscopy (FCS). Conformational fluctuations of the DNA move the bound fluorophores stochastically into the diffraction-limited excitation volume of a focused laser beam in a confocal microscope. From the time correlation functions of the fluorescence intensity, we obtain the mean square displacement of the DNA on a time scale from microseconds to seconds. We see a substantial decrease in the power spectral density of the displacement fluctuations at frequencies below 10 Hz in the dead cells, compared to the live cells. The larger fluctuations in the living cells may indicate that the fluctuations on this time scale may be driven by active processes involving molecular motors that generate forces by ATP hydrolysis. On shorter time scales, we see little difference between live and dead cells, suggesting that processes on corresponding short length scales rely primarily on thermally-driven diffusive mechanisms.

### Transcription

**3010-Pos Board B165**

**Roles of Pyrophosphorylase in Transcription Initiation**

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RNA polymerase can cleave NMP at 3'end of nascent RNA by adding pyrophosphate (PPi) to the NMP, and produces NTP. This reaction called pyrophosphorolysis is a chemically reverse reaction of RNA elongation. During transcription-competent open promoter complex. Although much progress has been made in the study of transcription initiation by *E. coli* RNA polymerase (RNAP), many questions still remain regarding the “isomerization” steps which open the DNA and stabilize the transcription-competent open promoter complex. Large scale conformational changes in RNAP and promoter DNA occur, and allosteric communication occurs over large distances, but the details are poorly understood. are these mechanistic steps universal or promoter-specific? How do they relate to transcriptional output? To address these questions, we are determining the kinetics of transcription initiation and transcription product distribution at the majority of bacteriophage promoters T7A1 and AP, which exhibit very different kinetic and transcriptional behavior, as well as several variants of both. These promoter sequences differ greatly in the “discriminator” region between the −10 element and the transcription start site. The non-template strand of the discriminator region is known to interact with region 1.2 of the RNAP σ subunit, stabilizing the open promoter complex. Our results show that this interaction is coupled to the assembly of RNAP downstream mobile elements (DME) on duplex promoter DNA. Together, σ 1.2-discriminator interactions and DME assembly increase lifetimes of open complexes up to 10^5-fold and control the RNA product distribution (i.e., short abortives versus long transcripts). The DME are also targets of regulation by proteins such as T7 gp2, and are proposed to be involved in interactions between RNAP and far upstream DNA which affect the kinetics of putting the downstream duplex in the active site cleft and opening it. These studies show that discriminator interactions with σ 1.2 control the structure, lifetime, and transcriptional outputs of the final open complex, suggesting that the discriminator region may be critically important for transcription regulation in the cell.

**3013-Pos Board B168**

**Mechanisms of Na⁺/H⁺ Exchanger Isoform 3 Gene Proximal Promoter Modulation by Parathyroid Hormone**

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Previous studies have shown that parathyroid hormone (PTH) chronically inhibits NHE3 in opossum kidney cells (OKP) by reducing both, total NHE3 protein and mRNA levels due to transcription modulation. The purpose of this study was to elucidate the inhibitory mechanisms of PTH on the NHE3 gene promoter. Different segments of the proximal promoter (~157/+31; −85/+31; −65/+31 and −44/+31) were inserted in the pGL3-Basic luciferase reporter vector. OKP cells were transiently transfected with the vector constructs and kept in serum-free media for 24 h. Cells were then treated with 10-7M PTH for 24 h and promoter activity was determined. These segments were also evaluated by gel shift assays (GMSA). Nuclear expression of the Sp3/EGR-1 transcription factors was also assayed after treatment with 50 μM 1, 2, 3, 4, 5, 6-hexabromocyclohexane (JAK2 autophosphorylation inhibitor), 10 μM Stat3 inhibitor (TAK1 inhibitor) 1 μM KT5720 (PKA inhibitor) for 24 h. PTH decreased the promoter activity of the fragments −65 and −44 (~24% and 29% respectively). Inhibition of JAK/STAT and PKA pathways abolished this suppressor effect of PTH. In GMSA, PTH reduced the protein-DNA affinity of the segment −44/+31. Western blot analysis of the transcription factors (Sp3/EGR-1) after treatment with PAK and JAK/STAT signaling pathways inhibitors showed that alone, these inhibitors did not affect their nuclear expression. In conclusion, these data suggest that the cis-element(s) required for PTH responsiveness must be localized in the proximal promoter; the JAK/STAT and PKA pathways may be involved in this inhibitory response; and this effect of PTH on promoter transcription is probably due to changes in protein-DNA affinity without altering transcription factors nuclear expression.

**3014-Pos Board B169**

**Molecular Dynamics Study of RNA Polymerase Transcription from Free Energy Calculation**

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Genetic transcription lies at the heart of important biological processes. Despite great experimental progress being made in recent years, detailed structural and physical mechanisms remain elusive. In this work, we chose a single-subunit RNA polymerase (RNAP) from bacteriophage T7 as a model system. Translocation of the RNAP along DNA has been studied by both experimental and computational work. Single molecule experiments detected a small free energy bias between pre- and post- translocation states of T7 RNAP. Our previous modeling suggested that the translocation energy bias aids nucleotide selection at the pre-insertion state. In current work, we are testing hypotheses...
made from the previous study and investigate conformational changes at atomistic details. Free energy changes involved in translocation process are calculated from molecular dynamics simulations. In particular, O-helix in the finger domain and Tyr<sup>507</sup> residue near the active site are supposed to play important roles during each elongation cycle. Correspondingly, we examine closely the energy cost of Tyr<sup>507</sup> fluctuating IN and OUT of the active site, as well as that of opening and closing of O-helix. Physical insight of polymerase transcription can be gained further by combining our current free energy studies at atomistic scale with former kinetic modeling that links to experimental research.

3015-Pos Board B170
Simulation Studies on Nucleotide Selection in T7 RNA Polymerase Transcription
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Transcription by RNA polymerases (RNAPs) is essential in gene expression. During transcription elongation, an incoming RNA nucleotide binds to the catalytic center of the polymerase, and is added to the end of an existing transcript when the nucleotide correctly pairs with a template DNA nucleotide. Accurate selection of NTPs is key to maintaining high transcription fidelity. Detailed mechanisms on how the nucleotides are selected are still lacking. Using T7 RNAP as a model system, we calculate binding affinities of various NTP molecules with the RNAP elongation complex, in both its insertion and pre-insertion states. Stable complex structures are obtained by using molecular dynamics simulation. Techniques such as MM-GB/SA, QM/MM-GB/SA are applied to compare the NTP binding affinities. In addition, free energy perturbation and thermodynamic integration methods are also utilized to calculate the rNTP binding free energies. Based on the simulation results, we would be able to decide which checkpoints (pre-insertion and/or insertion state) the RNAP utilizes essentially to select against wrong nucleotides. We would also like to know how each selection proceeds (rejection and/or inhibition), as well as how strong each selection is in terms of the binding free energy differences between right and wrong nucleotides.

3016-Pos Board B171
Transcription on Tangled DNA
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Bending and torsional deformations of DNA often occur in vivo and are important for various biological functions in the cell. In particular, DNA supercoiling (a combination of bending and torsional stresses within DNA) plays a central role in regulating gene expression. Recent experimental studies of short circular DNA fragments suggest that combined DNA torsional and bending stresses strongly impact transcription by RNA polymerase (RNAP). However, despite phenomenological observations establishing a relationship between DNA supercoiling and transcription kinetics, many details of the structure-function relationship between RNAP and DNA template mechanics remain unclear. We aim to understand how supercoiled DNA affects the structure of DNA-bound RNA polymerase, with the goal of specifically identifying protein domains that are sensitive to mechanically stressed DNA templates. Massively parallel molecular dynamics simulations are performed to describe the interaction of bacteriophage T7-RNAP with three DNA mimics possessing qualitatively distinct states of torsional stress: underwound, overwound, and relaxed. The simulations reveal the topologies of the minicircles in complex with T7-RNAP and the structural details of bent and twisted protein-bound DNA. We observe remarkable differences between the conformations of T7-RNAP in complex with underwound, overwound and relaxed minicircle templates. In particular, on overwound DNA, T7-RNAP is capable of transitioning from the classical elongation complex structure into a stable intermediate that resembles the structure immediately preceding elongation. This finding suggests a structural mechanism by which transcription elongation may be hindered on bent and overwound DNA templates.

3017-Pos Board B172
Direct Measurements of Transcription under Torsion
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During transcription, both RNA polymerase (RNAP) and the nascent RNA chain need to rotate relative to DNA at a speed of approximately a few hundred rounds per minute. The resulting accumulation of torsional stress cannot be dissipated immediately, and is therefore able to impact the motion of the RNAP. Despite the fact that torque is an essential aspect of transcription, the extent of its impact remains unknown. By performing single molecule measurements using an angular optical trap, we directly measure the torque that RNAP can generate as well as the transcription rate under torque. This approach provides a framework for investigating the influence of torque on various DNA-based translocases.

3018-Pos Board B173
Single Molecule Characterization of RNA Polymerase I: Force Free Kinetic Measurements
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RNA polymerase I (Pol I) exclusively transcribes the ribosomal RNA (rRNA) which constitutes the vast majority of the total cellular transcription products. Furthermore, rRNA synthesis is highly regulated due to cell division, growth and biogenesis. Previous studies demonstrated that there is a high correlation between rRNA level and the rate of protein synthesis in the cell [1, 2]. Therefore, the activity of Pol I is highly regulated during the cell cycle because of the energetic cost of protein synthesis. However, the precise activity and the mechanistic pathways of Pol I during transcription are still unknown. Here, we investigate the kinetics of Pol I transcription using the Tethered Particles Motion (TPM) technique. Unlike optical and magnetic tweezers, TPM allows observation of transcription in the absence of tension applied to the DNA template [3]. We also verified that the Upstream Activation Factor (UAF) is not required for basal in vitro transcription, in coherence with previous reports [4], transcription did not occur without TATA Binding Box Protein (TBP) and core factor (CF) proteins. We measured transcription rates, at different nucleotide concentrations, which are comparable with those previously reported from in vitro bulk studies. References:


3019-Pos Board B174
Flexibility in Transcription Start-Site Selection by RNA Polymerase Involves Transcription-Bubble Expansion (“Unscrunching”) or Contraction (“Unscrunching”)
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RNA polymerase (RNAP) is a molecular machine that carries out a series of reactions during transcription initiation: (i) RNAP binds to promoter DNA, yielding an RNAP-promoter closed complex (RPe), (ii) RNAP unwinds ~13 base pairs of promoter DNA surrounding the transcription start site, forming a single-stranded region (“transcription bubble”), and yielding an RNAP-promoter open complex (RPo), (iii) RNAP begins synthesis of an RNA product as an RNAP-promoter initial transcribing complex (RPTC), (iv) After RNAP synthesizes an RNA product ~11 nt in length, RNAP breaks its interactions with the promoter, escapes from the promoter, and begins transcription elongation as an RNAP-DNA elongation complex (RDe).

It has been known for four decades that the transcription start site can vary over a range of at least 5 bp—comprising the default start site (position +1), downstream-shifted start sites, (positions +2 and +3), and upstream-shifted start sites (positions −2 and −1)—and that the transcription start site can be reprogrammed within this range by the use of appropriate ribodinucleotide primers. However, the mechanistic basis of this flexibility in transcription start-site selection has not been known.