339-Pos Board B139
Determination of the DNA Binding Mode of the Transcriptional Repressor ICER
Mark D. DeMilio, Franklin E. Paulino, Carlos A. Molina, Johannes P.M. Schelvis.
Inducible cAMP Early Repressor (ICER) is a leucine zipper protein, a product of the cAMP Responsive Element Modulator (CREM) gene. ICER functions as a transcriptional repressor and its expression is abnormal in certain types of cancer where ICER acts as a tumor repressor. ICER binds to cAMP Responsive Elements (CRE's) found in the promoter sequences of genes involved in cellular growth. It also binds to the four CRE sites on its own promoter, thereby regulating its own expression. These four sites located on the CREM gene promoter are designated CARE-1 through CARE-4, and each one consists of 8 to 9 bases. On the basis of the presence of four CRE sites, we hypothesize that ICER may autoregulate its expression by cooperative binding to its own promoter. To test this hypothesis, we are performing titrations of purified ICER with double stranded DNA that is labeled with a fluorophore-quencher pair and monitor the binding by measuring the change in fluorescence due to an increase in resonance energy transfer upon a decrease in fluorophore-quencher distance. The dsDNA contains one or more of the CARE sites. We are determining the dissociation constant of ICER for each of the four CARE sites as well as examine the potential for cooperative binding between multiple CARE sites. Experiments on dsDNA (35 bp) with CARE-1 and/or CARE-2 have each yielded a dissociation constants of $k_d = 0.16 \pm 0.02$ μM, and no obvious evidence for cooperative binding between CARE-1 and CARE-2 has yet been observed. Analysis of dsDNA with CARE-3 and CARE-4 as well as for the entire promoter region with all four CARE sites will be presented and a model for binding of ICER to its own promoter will be discussed.

340-Pos Board B140
Tightly Looped DNA Represses Transcription Initiation by T7 RNA Polymerase
Troy A. Lionberger, Ankit Vahia, Craig T. Martin, Edgar Meyhöfer.
DNA looping by gene repressors has long been recognized as a widely conserved paradigm for prokaryotic repression. Yet, the extent to which the mechanics of the looped DNA itself plays a direct, physical role in repressing RNA polymerase (RNAP) remains debated. Recently, we have shown that transcription elongation by the bacteriophage T7 RNAP (T7RNAP) is significantly repressed from tightly looped DNA templates (*Biophys.J.*99(4),2010). However, it remains unclear if mechanically stressed DNA templates also affect RNAP activity in other phases of transcription. During transcription initiation, a polymerase bound to a looped DNA template encounters a fundamentally mechanical challenge: it must transcribe the first ~8-10 nucleotides of the template while maintaining contacts with the upstream promoter. Consequently, the polymerase must overtwist the torsionally constrained DNA template by nearly a full turn before it releases the promoter. We hypothesize that the mechanical properties of tightly looped DNA alone can be repressive to this process. To test this hypothesis, we have developed an assay that is capable of monitoring transcription initiation from DNA minicircle templates that sustain well-characterized amounts of bending and torsional stresses. Preliminary data suggests a substantial increase in the formation of abortive products from a 100-bp DNA minicircle compared to its linear counterpart. We interpret this observation to confirm our broad hypothesis that template mechanics can directly repress initial transcription. To determine if the observed repression is owed to the sharp bending curvature of the looped template or the torsional properties of the minicircle, we have generated minicircle templates that are untwisted, overtwisted, and nicked (and hence sustain no torsional stress). The ongoing characterization of these minicircle templates is expected to shed light on the molecular mechanism through which mechanical stresses in DNA templates can directly repress transcription initiation by T7RNAP.

341-Pos Board B141
Single-Molecule Studies of Human RNA Polymerase Ii Transcription Initiation: Core-Promoter Recognition
Alexandros Pertsinidis, Sang Ryul Park, Robert Coleman, Andrei Revyakin, Robert Tjian, Steven Chu.
We have developed a robust experimental platform that allows us to study the assembly dynamics of complex, multi-components biochemical systems at the single-molecule level. A key element of our implementation was the refinement of surface passivation protocols for single-molecule experiments, to achieve a 10- to 30-fold reduction in non-specific interactions for the various components of the Human RNA Polymerase II machinery. The reduced background of this optimized system allowed us to monitor the assembly of fluorescent transcription machinery components on promoter-containing DNA molecules, using the mutli-color (sub-)nanometer co-localization capabilities of our recently developed actively-stabilized microscope setup (Pertsinidis et al., Nature 2010).
In addition, we applied single-molecule spectroscopy tools, and as well as numerical modeling, to start dissecting the cascade of conformational rearrangements during Pre-initiation Complex (PIC) formation. Comprised of the General Transcription Factors (GTFs) TFIID/A/B/F/E/H and RNAPol II, assembly of this >50-polypeptide, Mega-Dalton complex commences with recognition of core-promoter elements by the TFIID complex. We observe that the TATA-binding (TBP) sub-unit of TFIID binds the consensus AdMLP core promoter with a transition to the severely kinked/partially unwound TATA-box state. Interestingly, we observe that TBP can also recognize variant TATA elements, resulting in structures with intermediate degrees of DNA deformation. Finally, our observations reveal additional conformational changes upon TFIIA (and to some extent TFIIB) recruitment, suggesting a mechanism for regulating further progression of the PIC assembly on strong as well as weak TATA-containing promoters but not on random DNA sequences.

342-Pos Board B142
Real-Time Initial Transcription by a Multisubunit RNA Polymerase
Kristofer Gryte, Thorben Cordes, Alexandra Tomescu, Ling Hwang, **Achillefs Kapanidis**.
Abortive initiation, the repetitive synthesis of short RNA transcripts before promoter escape and elongation, is a highly dynamic process that can constitute the rate-limiting step for many gene promoters. Previous studies using single-molecule Förster Resonance Energy Transfer (smFRET) demonstrated that abortive initiation occurs via a DNA-scrunching mechanism mediated by RNA polymerase. While these studies, along with biochemical work, have yielded insight into abortive initiation, no single-molecule method has been able to directly capture RNA synthesis/release, or the associated conformational changes of the transcription complex. Here, we descibe a highly sensitive smFRET assay that, for the first time, monitors transcription bubble expansion and compaction during abortive RNA synthesis and release in real-time; we also performed similar analysis using an assay that reports on the movement of downstream DNA toward the RNA polymerase main channel.
Using single-molecule time trajectories, we have observed multiple cycles (up to 50) of RNA synthesis by single RNA polymerase molecules and we used them to obtain the rates of RNA synthesis and release, as well as to study the dependence of the rates on nucleotide concentration and promoter sequence. Our initial results revealed that, surprisingly, the rate of nucleotide addition in initial transcription is 5-10 fold faster than elongation; moreover, we observed large heterogeneity between different RNA polymerase molecules with regards to specific steps in initial transcription; such heterogeneity may contribute to the noise associated with gene expression.

343-Pos Board B143
Promoter Opening-Closing Dynamics of Mitochondrial RNA Polymerase
Hajin Kim, Guo-Qing Tang, Smita S. Patel, Taekjip Ha.
Yeast mitochondrial (mt) RNA polymerase (RNAP) is an intriguing enzyme in that, while the polymerase Rpo41 is homologous to the single-subunit T7/T3 RNAP, its transcription factor Mtf1 appears to function similarly to the initiation factors of multi-subunit RNAPs. Yet, Mtf1's primary structure does not bear any similarity to Eukaryotic transcription factors. Fluorescence quenching measurement of each base (1) and DNA-protein cross-linking study (2) have demonstrated that Mtf1 facilitates the promoter melting and traps the non-template strand by direct interaction with the DNA. However, the dynamics of polymerase/factor binding and promoter melting are not well understood. Using single molecule techniques, we found that the complex of template DNA with Rpo41/Mtf1 undergoes opening-closing transitions without the proteins leaving the complex. Adding ribonucleotides decreased the closing rate for the cognate nucleotide but not for non-cognate nucleotides, implying that only the cognate nucleotide can stay long enough in the pre-initiation complex and stabilize the complex for accurate transcription. Rpo41 alone without Mtf1 can form the open complex on mismatched pre-melted template and Mtf1 further stabilizes this at open state by ~50-fold. Single molecule studies provide insights into the mechanism of promoter recognition and opening by cooperative action of the polymerase and the factor.
(1) G. Tang, S. Paratkar, and S. patel, "Fluorescence Mapping of the Open Complex of Yeast Mitochondrial RNA Polymerase", J. Biol. Chem. 284, 5514 (2009)
(2) S. Paratkar and S. patel, "Mitochondrial Transcription Factor Mtf1 Traps the Unwound Non-template Strand to Facilitate Open Complex Formation", J. Biol. Chem. 285, 3949 (2010)