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Probing The Structure And Function Of Transcription Complex Of RNA Polymerase II With TFIIF At Single Molecular Level
Wei-hau Chang.
Academia Sinica, Taipei, Taiwan.
Previously, we have developed a bio-conjugation method that allows us to specifically label the C-terminus of any subunit of a RNA polymerase. Here, we reconstitute a RNA polymerase II (Pol II) with TFIIF (IIF), in which the C-terminus of a RNA polymerase subunit is labeled with Cy3 and an amino acid of the largest subunit of TFIIF is labeled with Cy5. By measuring FRET at single molecular level, we are able to probe the structure information of Pol II/IIF in the absence of X-ray structure. By using single molecular FRET and tethered particle motion method, we are also carrying out transcription of Pol II in the presence or absence of IIF to identify the role of IIF in RNA elongation at various stages.

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Agaerye
Timothee Lionnet[1], Jeffrey A. Chao[1], Yaron Shav-Tal[2], Xavier Darzacq[3], Robert H. Singer[1].
[1]Albert Einstein College of Medicine, Bronx, NY, USA, [2]The Mina & Everard Goodman Faculty of Life Sciences & Institute of Nanotechnology, Bar-Ilan University, Ramat-Gan, Israel, [3]Ecole Normale Superieure - CNRS UMR 8541, Paris, France.
In order to ensure stable expression of an endogenous protein, the cell has to regulate each step of gene expression. Starting with transcription, we here observe in live cells the real-time transcription of an endogeneous gene, β-actin. To achieve this, we use a transgenic mouse model where RNA stem loops knocked into the untranslated region of β-actin mRNA are bound to a fluorescent MS2 reporter protein.
We monitor β-actin transcription levels in single fibroblast cells, either as a response to serum induction or in conditions of basal expression (constant serum level). This way, we are able to describe the kinetics of β-actin transcription over a long period. We observe that the cell responds to serum induction within minutes, and falls back to basal levels after ~1h, in agreement with data on fixed cells. We then compare how levels of transcription vary within one cell (between the alleles), and from cell to cell within a cell population. These findings uncover how extrinsic causes (influence of the environment within the cell) and intrinsic causes (inherent stochasticity of transcription at one gene locus) respectively contribute to genetic expression noise of an essential endogeneous gene. Supported by NIH grant EB2060.

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Metal Preference At The Second Metal Binding Site Of E. coli NikR
Christine M. Phillips, Paul S. Nerenberg, Catherine L. Drennan, Collin M. Stultz.
Massachusetts Institute of Technology, Cambridge, MA, USA.
Escherichia coli NikR (EcNikR) regulates cellular nickel uptake by binding free nickel ions at high affinity metal binding sites in the protein, which induces EcNikR binding to the *nik* operon - a process that leads to suppression of the gene encoding the nickel uptake transporter, NikABCDE. A structure of the EcNikR-DNA complex suggests that a second metal binding site is present in addition to the high affinity sites, and raises the question of which metal occupies this second site under physiologic conditions: K^+, which is present in the crystal structure, or Ni^{+2}. To determine which ion is preferred at the second metal binding site, and the reason for any preference of one ion over another, we calculated the electrostatic free energy of EcNikR binding to DNA with either K^+ or Ni^{+2} in the second site. While the interaction between EcNikR and DNA is more favorable when the second site contains Ni^{+2}, the large desolvation penalty associated with moving Ni^{+2} from solution to the relatively buried second site offsets this favorable interaction. Consequently, our data suggest that EcNikR binding to DNA is more favorable when the second site contains a K^+ ion. Moreover, additional calculations indicate that the second site is best suited for an ion having the size of K^+ and not Ni^{+2}, suggesting that the second site is optimized for K^+. Taken together, our data suggest that the second metal binding site contains K^+ and explain why K^+ is preferred over Ni^{+2} at this site.

298-Pos Board B177
Force-Dependence of Lac-Repressor Mediated DNA Loop Formation
Yih-Fan Chen, Gerhard Blab, David P. Wilson, **Jens-Christian Meiners**.
University of Michigan, Ann Arbor, MI, USA.
Protein-mediated DNA looping is a ubiquitous motif in transcriptional control schemes. Formation of these loops is driven by thermal fluctuations of the substrate DNA, which in turn are known to be exquisitely sensitive to mechanical constraints on the DNA. Because DNA in vivo is subject to a complex micromechanical environment, it is intriguing to study the effect of mechanical tension in the substrate DNA on the formation of these regulatory loops to investigate the role of mechanics in controlling gene regulation. For this purpose, we measured the formation and breakdown rates of lac repressor-mediated DNA loops under tension using constant-force axial optical tweezers.
We observed that an incremental force of less than 100 femtonewtons is sufficient to reduce loop formation rate about sevenfold in a construct with an interoperator spacing of 305 bp. This result suggests the possibility of mechanical pathways to control gene expression with forces that are two orders of magnitude lower than other typical intracellular forces acting on DNA, such as the forces exerted by RNA polymerase and molecular motors. Moreover, we developed a model that quantifies the relation between the force sensitivity of the loop formation rate and the angle between the incoming and outgoing DNA strand in the loop as a way to infer loop topology from our micromechanical measurements. We conclude that the LacI-mediated DNA loop prefers an anti-parallel loop topology over a parallel conformation.

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Malleable machines in transcription regulation: the Mediator complex
Ágnes Tóth-Petróczy[1], István Simon[1], Christopher Oldfield[2], Yuichiro Takagi[3], Keith Dunker[2], Vladimir Uversky[4], **Monika Fuxreiter**[1].
[1]Institute of Enzymology, BRC, Hungarian Academy of Sciences, Budapest, Hungary, [2]Center for Computational Biology, Indiana University, Indianapolis, IN, USA, [3]Department of Biochemistry and Molecular Biology, Indianapolis, IN, USA, [4]Institute for Intrinsically Disordered Protein Research, Indiana University, Indianapolis, IN, USA.
The Mediator complex provides an interface between gene-specific regulatory proteins and the general transcription machinery including RNA polymerase II (RNAP II). The complex has a modular architecture and cryo-EM analysis suggested that it undergoes dramatic conformational changes upon interactions with activators and RNAP II. These rearrangements were proposed to play a role in the assembly of the pre-initiation complex and also contribute to the regulatory mechanism of Mediator. In analogy to many regulatory and transcriptional proteins, we reasoned that Mediator might also utilize intrinsically disordered regions (IDRs) to facilitate structural transitions and transmit transcriptional signals. Indeed, a high prevalence of IDRs was found in various subunits of Mediator from both *Saccharomyces cerevisiae* and *Homo sapiens*, especially in the Tail and the Middle modules. The level of disorder increases from yeast to man, although in both organisms it significantly exceeds that of multi-protein complexes of similar size. IDRs can contribute to Mediator's function in three different ways: they can serve as target sites for partners with variable structure; they can act as malleable linkers connecting globular domains that impart modular functionality on the complex; and they can also facilitate assembly and disassembly of complexes in response to regulatory signals. Short segments of IDRs, termed molecular recognition features (MoRFs) distinguished by a high protein-protein interaction propensity, were identified in 16 and 19 subunits of the yeast and human Mediator, respectively. In *Saccharomyces cerevisiae* the functional roles of 11 MoRFs have been experimentally verified and those in the Med8/Med18/Med20 and Med7/Med21 complexes were structurally confirmed. The arrangement of disordered regions and that of the embedded interaction sites are similar in *Saccharomyces cerevisiae* and *Homo sapiens* yet their sequences are weakly conserved. All these data suggest an integral role for intrinsic disorder in Mediator's function.

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Structure/function Correlations in P. aeruginosa DNA Ligase LigD
Aswin Natarajan[1], Hui Zhu[2], Pravin A. Nair[2], Stewart Shuman[2], Ranajeet Ghose[1].
[1]City College of New York and Graduate Center, CUNY, New York, NY, USA, [2]Sloan-Kettering Institute, New York, NY, USA.
The ATP-dependent DNA ligase D (LigD) performs a major role in non-homologous end-joining (NHEJ) pathway. *Pseudomonas aeruginosa* LigD (PaeLigD) contains a N-terminal phosphoesterase domain (PE) domain followed by a ligase domain and a C-terminal polymerase domain. The PE domain (187 residues) possesses manganese dependent phosphodiesterase and phosphomonoesterase activities as it sequentially removes the 3′-ribonucleoside from the primer strand of the RNA primer-DNA template duplex and subsequently hydrolyzes the 3′-PO_4 to a 3′-OH group (1).
PaeLigD-PE belongs to a class of unique 3′- end-processing enzymes as it cleaves the primer strand to a point at which a single ligatable ribonucleotide remains (2).
Extensive mutagenesis studies have identified critical residues required for ribonuclease and 3′-phosphatase activities (1). Multiple active sites present in