1461-Pos Board B231
Binding and Translocation of Termination Factor Rho Studied at the Single-Molecule Level
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Rho is an essential hexameric helicase responsible for terminating 20-50% of all mRNA synthesis in *E. coli*. We used single-molecule force spectroscopy to investigate Rho-RNA binding interactions at the Rho-utilization (*rut*) site of the λtR1 terminator. Our results are consistent with Rho adopting two states, one involving Rho binding to 57 ± 2 nucleotides of RNA across all of its six primary binding sites, and the other binding 85 ± 2 nucleotides at these primary sites as well as a secondary site at the center of the hexamer. We supply direct evidence that Rho translocates 5′→3′ by a tethered-tracking mechanism, which may influence the ability of ribosomes or other factors to regulate RNA polymerase. Our findings allow us to develop a general model of Rho binding and translocation, and establish an experimental approach that will facilitate additional studies of termination by Rho and its regulation by transcription factors.

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Architecture of the Mediator Head Module
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Mediator is a key regulator of eukaryotic transcription, connecting activators and repressors bound to regulatory DNA elements with RNA polymerase II (Pol II). In the yeast S. cerevisiae, Mediator is organized into three modules (Head, Middle/Arm and Tail) that together comprise 21 subunits with a total mass over 1 MDa. Our understanding of Mediator assembly, and of its role in regulating transcription, has so far been hampered by limited structural information. By using state-of-the-art protein complex engineering and phasing methods, we solved the structure of the Mediator Head module (7 subunits, 223 kDa) at 4.3 angstrom resolution. Our structure reveals three distinct domains (Fixed jaw, Movable jaw, and Neck) that come together to form a flexible central joint region. Strikingly, we discovered a novel and elaborate structure in the Neck domain, in which a total of ten helices from five different subunits form a large multi-helical bundle as a single structure unit. An intricate pattern of interactions within this helical bundle ensures stable assembly of the Head subunits and provides binding sites for general transcription factors (GTFs) and Pol II. Mapping previously identified genetic mutations onto our Head module X-ray structure suggests that the Head module may juxtapose TFIIH and the carboxyl-terminal domain (CTD) of the largest subunit of Pol II (Rpb1) to facilitate CTD phosphorylation. These results reveal architectural principles underlying the role of Mediator in the regulation of gene expression.

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Fatty Acid Regulation of Enteric Infectious Disease
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Bacterial infections of the digestive tract afflict billions of people each year. These include infections such as travelers' diarrhea, cholera, and salmonella. The gram negative bacteria that cause these infections all function by using a genetic cascade to control the expression of virulence factors, and thus control infection. In the bacterium *Vibrio cholerae*, this process is controlled by a member of the AraC/Xyls transcription factor family, ToxT. Results from our lab, including the crystal structure of ToxT, have shown that ToxT is capable of binding a fatty acid, causing a conformational change and preventing DNA binding. This conformational change suppresses the virulence cascade in *V. cholerae*. Because many other AraC/XylS family members also control virulence cascades, we have begun an examination of the mechanism of action of these related proteins in hopes of discovering a common effector. We are using a combination of computational and biochemical methods to determine if fatty acids could function to repress other AraC/XylS family members and thus repress the virulence cascade in a variety of infectious bacteria.

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Detection of Specific mRNA Synthesis in Rotavirus using Single Molecule Hybridization
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Rotavirus is the main cause of severe gastroenteritis in humans and animals. The capsid encloses 11 highly packed segments of double stranded RNA (dsRNA) and 12 transcription polymerases complexes arranged along 12 vertices of the icosahedral capsid. Inside the cell it becomes transcriptionally active and synthesises positive single stranded RNA (ssRNA). The synthesis occurs after a few seconds of setting transcription conditions. The newly ssRNAs are released simultaneously through twelve specialised channels, but the detection and assignment of specific segments to the channel (if any) is unknown with current standard microscopy techniques. A detailed description of this process is needed to understand the model of transcription of the virus, its genomic packaging and the assembly of the virus.
Here, we use total internal reflection fluorescence combined with alternating laser excitation (TIRF-ALEX) microscopy and single molecule hybridization to identify, for the first time, the synthesis and release of specific single-stranded RNAs on the surface of a rotavirus capsid. A FRET pair consisting of a single stranded DNA labelled with Cy3B (FRET donor) or ATTO647N (FRET acceptor) was designed to hybridize with complementary RNA segment 11 with an expected value of approximately 35%. Biotinylated capsids were then immobilised on neutravidin-treated slides, incubated with ssDNA fragments that hybridize with the viral RNA only in presence of nucleotides; control experiments included parallel hybridization experiments in the absence of viral transcription. Our smFRET results show molecular populations with the expected FRET value of single hybridisation events on the viral surface. These findings support a transcription model in which one ssRNA segment is associated with a single transcription complex and individual dsRNA segment is packed with a single polymerase complex. Our results exclude alternative models in which an individual segment is transcribed by several polymerases.

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Control of Gene Expression by Modulated Self-Assembly
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Numerous transcription factors self-assemble into different order oligomeric species [1] in a way that is actively regulated by the cell. Here, we use a novel quantitative framework to capture the effects of modulated self-assembly in gene expression [2]. We show that this mechanism provides precision and flexibility, two seemingly antagonistic properties, to the sensing of diverse cellular signals by systems that share common elements present in transcription factors like p53, NF-κB, STATs, Oct and RXR [2]. Applied to the nuclear hormone receptor RXR, this framework accurately reproduces a broad range of classical sets of gene expression data and corroborates the existence of a precise functional regime with flexible properties that can be controlled both at a genome-wide scale and at the individual promoter level. We show that key to the success of the computational framework is that it incorporates the regulatory complexity of RXR mediated signaling [1,3,4], which involve multiple DNA-binding sites at the promoters of retinoid targeted genes and the ability of RXR to precisely tailor the response to each individual gene depending on the arrangement of these sites.
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Transcriptional Proofreading in Dense RNA Polymerase Traffic
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The correction of errors during transcription involves the diffusive backward translocation (backtracking) of RNA polymerases (RNAPs) on the DNA. A trailing RNAP on the same template can interfere with backtracking as it progressively restricts the space that is available for backward translocation and thereby ratchets the backtracked RNAP forward. We analyze the resulting negative impact on proofreading theoretically using a lattice model of transcription introduced earlier [1]. Our analysis indicates that the efficiency of error correction is essentially determined by the rate for the initial backtracking step, while a high transcript cleavage rate ensures that the correction mechanism remains efficient at high transcription rates. Our analysis can also be applied to cases with transcription-translation coupling where the leading ribosome on the transcript assumes the role of the trailing RNAP.
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