Transition metal ions such as manganese (Mn) enable a multitude of cellular functions that are vital to life. In bacteria, Mn is considered universally essential for resistance and prevention of oxidative stress typically generated by a bacteria's exposure to reactive oxygen species (ROS). However, in excess, Mn can be toxic, leading to mis-metalation of metalloenzymes and regulatory transcription factors. Being a metal ion, Mn cannot be consumed by the cell unlike sugars and vitamins; it can only be transported by the cell. Hence, many bacteria like *Bacillus subtilis* have evolved metalloregulatory proteins, such as MntR, that regulate intracellular Mn concentration by both repressing the expression of uptake transporter proteins and activating the expression of efflux proteins in response to elevated Mn levels. While the role of MntR as a dual regulatory protein had been well established in prior studies, the molecular mechanism of transcriptional activation by the protein was not well understood. We used cryogenic electron microscopy (cryo-EM) to explore the molecular basis of gene activation by MntR and report a structure of four MntR dimers bound to four18-base pair sites across an 84-base pair regulatory region of the *mneP* promoter. Our structures, along with solution studies including mass photometry, fluorescence-based size exclusion chromatography and *in vivo* transcription assays, reveal that MntR dimers employ polar and non-polar contacts to bind cooperatively to an array of low-affinity DNA-binding sites. These results reveal the molecular basis for cooperativity in the activation of manganese efflux, furthering our currently limited understanding of bacterial Mn(II) homeostasis.

2721-Pos

BPS2025 - Impact of prenatal titanium dioxide nanoparticle exposure on DNA methylation and gene expression patterns in the brains of mice and neuronal cells

Saori Taneo, Atuto Onoda, Ken Tachibana.
Faculty of Pharmaceutical Sciences, Sanyo-Onoda City University, Sanyo-Onoda, Yamaguchi, Japan.

Titanium dioxide nanoparticles (TiO_2-NP) are widely used nanoparticles in industry. Currently, exposure to TiO_2-NP during pregnancy may cause some toxic effects on the growth of the central nervous system in mouse offspring. Previous research suggests TiO_2-NP exposure can disrupt brain development, but the underlying mechanisms are not yet identified. In this study, we analyzed the impact of prenatal TiO_2-NP exposure on global DNA methylation and mRNA expression patterns in the brains of neonatal mice and neuronal cells. We analyzed the effects of prenatal TiO_2-NP exposure on the DNA methylation status in brain samples of mouse offspring using a CpG island microarray. GSEA analysis using GO terms suggested that genes are related to cell proliferation, organism development, and regulation of transcription factors. GSEA analysis using MeSH terms indicates that these phenomena are associated with the proliferation and differentiation of neural stem cells. It can be speculated that prenatal TiO_2-NP exposure adversely impacts brain functions, such as impairment of learning and memory or the dopaminergic system by disrupting the proliferation and differentiation of neural stem cells at the developmental stage. Now, we investigate and would like to discuss whether prenatal TiO_2-NP exposure alters the DNA methylation status of neural stem cells, and subsequently causes abnormal regulation of transcription factors that control development and differentiation. The findings contribute to a growing body of evidence that nanoparticles can induce potential long-term consequences for neurodevelopment by altering the DNA methylation status of fetal tissues, especially neural stem cells, in the brain. This research emphasizes the importance of understanding the risks associated with TiO_2-NP exposure during pregnancy and needs more investigation to determine the broader implications for human health.

2722-Pos

BPS2025 - Spatial organization of regulatory chromatin at transcription condensates

Ganesh Pandey[1], Jonah Galeota-Sprung[2], Alisha Budhathoki[1], Filmon Medhanie[1], Jan-Hendrik Spille[1,2].
[1]Department of Physics, University of Illinois Chicago, Chicago, IL, USA, [2]Department of Biomedical Engineering, University of Illinois Chicago, Chicago, IL, USA.

Transcription condensates compartmentalize important components of the transcription machinery, such as the mediator complex, the coactivator Brd4, and RNA polymerase II (Pol II). Inhibition of Brd4 binding to the active chromatin mark H3K27ac results in the dissolution of condensates. This suggests that active enhancer and promoter regions link transcription condensates to chromatin. But there are thousands of active chromatin regulatory elements in a cell whereas only a few condensates can be detected in a single cell nucleus. It remains unclear what sets apart chromatin structures at condensates and how condensates interact with chromatin structures. We use multicolor single-molecule localization microscopy to uncover how the active chromatin marks H3K27ac, H3K4me1, and H3K4me3 interact with transcription condensates. Our data reveal that active chromatin partitions into transcription condensates. Active promoter marks (H3K27ac, H3K4me3) are enriched toward the core of condensates while enhancer marks (H3K27ac, H3K4me1) are more likely localized to the surface. In contrast, inactive chromatin marks (H3K27me3) are depleted from transcription condensates. Furthermore, we find that several nanoclusters of active chromatin marks are associated with single condensates. This suggests that multiple active regulatory elements interact with condensates simultaneously. An analysis of functional states of Pol II corroborates these chromatin-level results. We find that the initiating phospho-isoform (Ser5p-Pol II) is highly enriched toward the core of condensates where we found the strongest promoter marks. The elongating phospho-isoform (Ser2p-Pol II) is detected in multiple distinct nanoclusters at each condensate, giving additional support for our interpretation that there are multiple simultaneously transcribed elements associated with each condensate. Our results shed new light on the organization of active chromatin at transcription condensates and their functional role in the coexpression of multiple genes.

2723-Pos

BPS2025 - Single-molecule visualization of the kinetic control of mammalian transcription elongation

Yukun Wang[1], Xizi Chen[2], Joel E. Cohen[3], Yanhui Xu[4], Shixin Liu[1].
[1]Liu Laboratory, The Rockefeller University, New York, NY, USA, [2]Fudan University, Shanghai, China, [3]Laboratory of Populations, The Rockefeller University, New York, NY, USA, [4]Institutes of Biomedical Sciences, Fudan University, Shanghai, China.

Transcription, the initial step of gene expression fundamental to all forms of life, involves several highly regulated phases. Among these, the elongation phase, where the bulk of RNA transcripts are synthesized, has emerged as a critical stage of transcriptional control. Defects in elongation are linked to human diseases including cancer. However, a detailed understanding of the kinetic control of transcription elongation is challenging due to the complex and dynamic interactions of many elongation factors that regulate the activity of RNA polymerase II (Pol II). To address this, we developed an in vitro reconstituted single-molecule system to visualize the transcription events of individual mammalian elongation complexes (ECs) on DNA templates of several kilobases in length. We were able to obtain unprecedented measurements of Pol II's translocation and pausing kinetics with different combinations of elongation factors. Using fluorescently labeled factors, we delineated the timing of factor binding in relationship with the speed change of the EC. Our findings reveal that mammalian Pol II possesses a low basal activity and its fast elongation requires several key elongation factors. Among those, PAF1C is critical to shifting Pol II into a higher gear, and its recruitment to the EC is dependent on the phosphorylation of Pol II by the P-TEFb kinase and is further stabilized by SPT6. Furthermore, RTF1 and phosphorylated DSIF are needed to cooperate with PAF1C to achieve maximal EC activity. Our study establishes a molecular framework for characterizing the kinetic regulation of eukaryotic transcription on chromatin.

2724-Pos

BPS2025 - Mechanisms and consequences of RNA duplex formation within the exit channel of RNA polymerases

Junqiao Zhu, Michael Z. Palo, Robert Landick, Aaron A. Hoskins.
Department of Biochemistry, University of Wisconsin Madison, Madison, WI, USA.

During the co-transcriptional folding of nascent RNA, the regulatory interplay between RNA polymerase (RNAP) transcription and nascent RNA structure is a ''two-way street.'' RNA structures forming within the RNA exit channel interact with the polymerase to influence RNA synthesis, while RNAP itself can impact RNA structure folding within the channel. Understanding this interplay enhances our knowledge of RNAP as a potential RNA-folding chaperone and the connection between transcription and RNA processing. To unravel this interplay, we combined both single-molecule and biochemical assays using purified polymerases assembled on