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poorly understood. We propose granularity results from the formation of co-existing phase separated pre-ribosomal assembly hubs whose spatial sequestration is regulated by ribonucleoprotein composition and ribosome assembly status. Seminal studies in the 1970s by Nomura and others (Nomura, Science, 1973), as well as Cryo-EM studies, demonstrated association of early binding r-proteins and/or nucleolar proteins to rRNA stabilize and induce conformations(s) conducive to ribosome assembly. Our *in vitro* condensate assays and superresolution cellular microscopy studies demonstrate binding of r-proteins and/or the disordered Arginine rich nucleolar protein SURF6 dramatically alters rRNA/NPM1 phase separation and promotes the formation of multiphase droplets mimicking the granularity of the GC. We propose competing protein/rRNA interactions lead to the granularity of the GC *via* a molecular hand-off mechanism. Broadly, these studies provide novel insight into how rRNA structure and RNP composition define the liquid-like behavior of the GC and suggest how mutations in NPM1 or r-proteins could alter nucleolar LLPS and allow for deregulation of ribosome biogenesis and pathogenesis.

1742-Pos

Liquid liquid phase separation packages cargo for their delivery to secretory storage granules

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Insulin is a key regulator of human metabolism, and its misfunction leads to diseases such as type2 diabetes (T2B). However, despite extensive research in the last three decades, it is still unknown how proinsulin is targeted from the trans-Golgi Network (TGN) to secretory storage granules as no cargo receptor has been identified. Chromogranin proteins (CGs) are central regulators of granule biosynthesis in multiple endocrine tissues; however, their molecular role is poorly understood. Here we show that CGs undergo liquid-liquid phase separation (LLPS) at low pH that mimics the TGN milieu. Divalent cations such as calcium seem to be dispensable for this process. Under these conditions, CG condensates recruit and sort proinsulin and other granule destined cargo molecules. Furthermore, we show that sorting at the TGN exclusively depends upon LLPS of CGs and cargo selectivity is largely based on the size of clients. Finally, we provide evidence that electrostatic interactions and the N-terminal intrinsically disordered domain of CGB facilitates LLPS, which is critical for granule formation. Based on these novel data, we propose that phase-separated CGs act as a ‘‘cargo sponge’’ within the TGN lumen, gathering soluble proteins into the condensate non-specifically, thus facilitating receptor-independent sorting of cargo molecules. These findings challenge the canonically held views concerning sorting at the TGN and provide new molecular insights into understanding granule biosynthesis in insulin-secreting β-cells.

Posters: Transcription

1743-Pos

DNA opening during transcription initiation by human RNA polymerase II in atomic details

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RNA polymerase II (RNAPII) is a macro-molecular complex that synthesizes RNA by reading the DNA code, a process called transcription. During the initiation step of transcription, RNAPII opens double-stranded DNA in order to read the DNA code. Since formation of the DNA transcription bubble remains poorly understood, we used molecular dynamics simulations to provide atomic-level insights into this crucial step of transcription. Here, by steering the simulations with a combination of (i) guided DNA rotation and (ii) path collective variables, we obtained for the first time continuous atomic trajectories of the complete DNA opening process. The simulations provide insights into the role of loop dynamics and protein-DNA interactions during DNA opening.

1744-Pos

Cryo-EM structures of riboswitches in paused elongation complexes

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Riboswitches are structural RNA elements mostly found within the untranslated regions of bacterial messenger RNAs (mRNAs) and play an important role in regulating gene expression at the level of transcription or translation. Here we use techniques of cryo-electron and single molecule microscopy in order to investigate the regulation of transcription termination mediated by the fluoride-sensing riboswitch. We expect to identify key interactions between the RNA polymerase and the riboswitch specifically within the active site and the RNA exit channel that will help to illuminate the mechanism of transcription termination. Single-molecule experiments, such as Single-Molecule Kinetic Analysis of RNA Transient Structure (SiM-KARTS) assays can expand on this mechanism by analyzing mutants of the identified key interactions on both RNAP and RNA and observing the effect on riboswitch structure. Our single molecule experimental setup allows for the investigation of riboswitch mechanisms in the context of the transcriptional machinery which is known to modulate RNA structure within a cellular context. We anticipate that these results will further explain the regulatory process by which the fluoride riboswitch modulates transcription efficiency and will improve our understanding of riboswitch dynamics, structure, and regulatory mechanism. Identifying the pivotal interactions between riboswitches and the transcription machinery has the potential to assist in designing new classes of anti-bacterial drugs.

1745-Pos

Positive supercoiling facilitates RNAP elongation past protein-mediated DNA loops

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Some proteins, such as the *lac* repressor (LacI), induce the formation of loops that alter DNA topology and generate torsional barriers. The supercoiling generated by elongating RNA polymerase may facilitate passage through such barriers. Tethered particle motion measurements of *E. coli* RNA polymerase (RNAP) progress along templates in conditions that prevented, or favored, LacI-mediated DNA looping revealed that RNAP paused longer at unlooped LacI obstacles, or those barring entry to a loop, than those barring exit from the loop. In addition, RNA polymerase transcribed a looped segment more slowly than an unlooped segment and paused more briefly at LacI obstacles on templates positively supercoiled by magnetic tweezers. Positive supercoiling propagating ahead of polymerase within a torsion-constrained DNA loop appears to facilitate dissociation of a LacI roadblock and, more generally, elongation along topologically-complex, protein-coated templates.

1746-Pos

Reconstitution of transcriptional pausing in human mitochondria

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Transcription in human mitochondria by mitochondrial RNA polymerase (mtRNAP) is coupled to mitochondrial DNA (mtDNA) replication, and the mechanism of how transcription switches to replication is not fully understood. Transcriptional pausing, which is predicted to occur upstream of this switch, could play an important regulatory role. My project aims to fill the gap in knowledge about the mechanism of transcriptional pausing by mtRNAP, its potential regulatory roles on human mtDNA and its effects on mtDNA replication. To accomplish this goal, I have successfully reconstituted transcription in vitro on DNA sequences encoding human mtDNA promoters and the downstream CSB regions. In these reactions, I observed paused RNA species corresponding to the positions identified by previous in vivo sequencing of pause sites. Going forward, I will elucidate the mechanism of mtRNAP pausing by in vitro transcription assays to test the nature of the pauses, use single-particle cyro-EM on mtRNAP in the paused state and 6-methylisoxanthopterin (6-MI), a fluorescent nucleotide analog, to monitor the translocation state of mtRNAP in the paused state.

1747-Pos

Single molecule activity assay for SARS-CoV-2 RNA dependent RNA polymerase

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Transcription and replication are crucial for cell survival and functionalization. In the case of viruses, regulation of transcription and replication determines proliferation and adaptation to the environment. Beta-coronaviruses have become a serious global health concern, causing several global epidemics, such as Severe Acute Respiratory Syndrome (2002), Middle East Respiratory Syndrome (2012) and most recently COVID-19 (2019). SARS-CoV-2, a beta-coronavirus and the pathogen of COVID-19, is a positive-sense single-stranded RNA virus. Like many other RNA viruses, SARS-CoV-2 relies on a single enzyme, RNA dependent RNA polymerase (RdRp) for its transcription

and replication processes. Due to its importance, RdRp is one of the main targets for therapeutic approaches. We developed an in-vitro, single-molecule based assay to detect the polymerization activity of the RdRp complex. Using this assay, we optimized the activity of the minimal SARS-CoV-2 RdRp (composed of nsp12, nsp7, and nsp8) by testing various conditions such as different concentrations of salt, molecular crowding agents, and divalent metal ions. The broad compatibility of our activity assay will enable the study of SARS-CoV-2 transcription and replication mechanisms and will be useful in the development of antiviral agents that inhibit the COVID-19 RdRp and potentially many other viral RdRps.

1748-Pos

Absolute quantitation of the yeast Whi5 transcriptional repressor during G1 phase growth

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To establish and maintain an homeostatic size optimized to their growth conditions, cells set and sense a critical size threshold for commitment to division in late G1 phase, known as Start in yeast. Start depends on the expression of over two hundred genes required for budding, DNA replication spindle pole body duplication, and other functions, collectively termed the G1/S regulon. The timing of Start is determined in part by two antagonistic proteins: an activator of Start transcription, Swi4, and its repressor, Whi5. It has been reported that dilution of Whi5 as cell grow controls the timing of Start while Swi4 concentration remains constant (1) or, conversely, that the concentration of Whi5 remains constant with increasing size in G1 cells while Swi4 concentration increases (2, 3). To resolve the discrepancy between these contradictory results, we used single cell imaging to quantify Whi5-GFP intensity as a function of time, rather than size under experimental conditions that largely eliminate confounding effects of photo-bleaching. No significant time-dependent change in Whi5-GFP intensity in G1 cells was observed. Moreover, deletion of one copy of the *WHI5* gene in a diploid strain decreased the nuclear Whi5 protein concentration two-fold as expected, yet led to only a small decrease in size. Together, these results strongly argue against a model where Whi5 dilution would be a critical determinant of Start.

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1749-Pos

Structural insights into the sequence preference of PU.1, a conserved helix-turn-helix transcription factor

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Winged helix-turn-helix (wHTH) proteins are ubiquitous DNA-binding motifs in all kingdoms of life. In eukaryotes, wHTH motifs are found in many transcription factors which recognize a wide range of cognate sequences; both high- and low-affinity binding being biologically important and non-interchangeable *in vivo*. To better understand the structural mechanisms through which wHTH containing proteins derive sequence selectivity, we are studying the ETS family of transcription factors. We focus on the protein PU.1 for structural examination owing to its broad range of DNA target preferences and affinities which set it apart from other ETS members. Despite a large inventory of wHTH/DNA complexes in the protein data bank, the majority of structures are bound to optimized sequences. The paucity of lower-affinity complexes leaves a gap in our current understanding of the structural basis of low-affinity binding. To address this, we have solved a suite of co-crystallographic PU.1/DNA complexes with altered affinities. Together with an optimal high-affinity sequence, we solved a low-affinity PU.1/DNA complex (the first such complex for any ETS member). To further explore the role of molecular hydration and DNA dynamics in target site selection, we also solved complexes with protein mutants which disrupted hydration properties and DNA sequences containing targeted minor-groove mutations that preserve protein/DNA contacts in the major groove. With a drastic improvement in resolution across our structures compared to the previously determined PU.1 DNA complex (2.10 to 1.28 Angstroms), sidechain dynamics, DNA motility, and previously unobservable shifts in interfacial hydration dictating binding affinity have come into focus. Overall, the structures suggest that the molecular determinants for sequence preference are strongly influenced by sidechain conformational dynamics. We expect these dynamics, together with coupled hydration, to significantly drive the selectivity of PU.1 for cognate DNA variants.

1750-Pos

Expression dynamics of G1 cyclins in *Saccharomyces cerevisiae* revealed by two photon scanning number and brightness microscopy

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Cell size homeostasis, which results from the coordination between cell growth and division, is a critical aspect of cell physiology and function in all species. The synchronization between these processes takes place in part in late G1 phase, termed Start in yeast, and marks irreversible commitment to division. Start timing depends heavily on the G1 cyclin, Cln3, which activates the Cdc28 cyclin dependent kinase. It is thought that Cln3-Cdc28 initiates Start by phosphorylation of the transcriptional repressor Whi5, causing its dissociation from the transcriptional activator complex, SBF, and export from the nucleus. SBF activates over two hundred genes in the Start regulon including two other cyclins, Cln1 and Cln2, which also phosphorylate Whi5 and thereby form a positive feedback loop that ensures a rapid and irreversible transition. However, the expression dynamics of Cln3 and the timing of the Cln1/2 feedback loop with respect to cell size and Start are not well characterized because of low Cln1/2/3 abundance and rapid turnover. Here, using two photon scanning number and brightness microscopy, we have examined the nuclear expression dynamics of the three G1 cyclins in single cells of *S. cerevisiae* strains expressing *CLN-GFP*mut3 fusions at their endogenous chromosomal loci. We provide a framework to interpret Cln1/2/3 expression dynamics in context of Start activation and reinforcement by positive feedback.

1751-Pos

A thermodynamic model of bacterial transcription

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Transcriptional pausing is highly regulated by the template DNA and nascent transcript sequences. Here, we propose a thermodynamic model of transcriptional pausing, based on the thermal energy of transcription bubbles and nascent RNA structures, to describe the kinetics of the reaction pathways between active translocation, intermediate, backtracked, and hairpin-stabilized pauses. The model readily predicts experimentally detected pauses observed in high-resolution optical tweezers measurements of transcription, in terms of pause sites, duration, and mechanisms. Unlike other models, it also predicts the effect of tension, the GreA transcription factor and RNase A on pausing.

1752-Pos

Mechanism-driven approaches towards synthesis of highly pure RNA for a wide variety of applications

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The impact of RNA in science has grown exponentially in the past three decades. In biology, alternative splicing, long noncoding RNAs, RNA editing, and riboswitches complement translation and transcription. RNA is also increasingly used in nanotechnology and medicine. Biophysical studies of RNA require high purity, yet RNA is still synthesized today as it was 30 years ago and is similarly characterized with gel assays that cannot assess a wide diversity of impurities. Although RNA analytics are only starting to catch up, we know that *in vitro* transcription, as currently practiced, introduces a range of heterogeneous impurities. This arises largely due to the accumulation of product in traditional high yield reactions. The product RNA rebinds the polymerase to template extensions to the originally encoded RNA. Our lab is now developing greatly improved approaches for RNA syntheses. In one type of approach, high salt inhibits RNA product rebinding, while constructs that strengthen the polymerase-promoter interaction rescue salt's inhibition of promoter binding (elongation is insensitive to salt). Relatively simple approaches can dramatically improve both the quality and yield of RNA, and the approaches apply equally to short and long RNAs. We are also developing flow synthesis approaches that remove RNA from the reaction as soon as it is formed, further preventing the conversion of correct product into (much) longer, partially double stranded RNA impurities. The end result is higher yields RNA with substantially higher purity.