Mechanism of assembly of an elongation-competent SARS-CoV-2 replication transcription complex

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DOI
10.1016/j.bpj.2022.11.1071

Publication date
2023

Document Version
Final published version

Published in
Biophysical journal

nonspecific binding sites. Such a proposal was also investigated through a highly simplified spherical protein model along with stochastic simulations, which well supported that flanking DNA sequences impact on overall protein dissociation kinetics and therefore on measuring binding affinity variations with specific or nonspecific DNA in the central binding site.

834-Plat
A single molecule TIRF-based platform to study DNA supercoiling effect on DNA processing enzymes
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DNA topology plays an important role in regulating DNA metabolic pathways. DNA supercoiling changes the DNA properties and proteins may differentially interact with DNA under various supercoiling states. A biophysical investigation of how proteins differentiate various topological states could further our understanding of protein-DNA interactions. We developed new single molecule total internal reflection fluorescence (smTIRF) -based platform to investigate how DNA topology affects protein-DNA interactions. We used this platform to study MutS, which detects a mismatched base pair to initiate mismatch repair, and CRISPR-Cas9. We generated plasmids that are site-specifically labeled with one or two fluorophores and a biotin. We prepared them in three different topological states: ''Linear'', ''relaxed circular'', and ''negatively supercoiled''. For MutS study, we designed a Cy5 labeled plasmid with an adjacent mismatch. We observed FRET between Cy3-MutS and Cy5 when MutS bound the mismatch. In the presence of ADP, MutS bound the negative supercoiled DNA with a longer residence time compared to the relaxed circular DNA, suggesting that negative supercoiling helps mismatch recognition. For CRISPR-Cas9, we created a dual labeled plasmid to observe R-loop formation induced by dCas9. R-loop formation rate was similar for all three DNA topological states when the guide RNA sequence matched the target DNA sequence, while the rate was highest for the negatively supercoiled DNA and decreased progressively for the relaxed circular DNA and the linear DNA when we introduced one mismatch in either the PAM (protospacer adjacent motif)-proximal position or the PAM-distal position. Our observation confirms that DNA negative supercoiling assists DNA unwinding by Cas9, and this can exacerbate off-target effects in the cell. Collectively, our smTIRF-based platform allows high throughput investigation of supercoiling effects on DNA protein interactions as demonstrated using two different biological systems.

835-Plat
Mechanism of assembly of an elongation-competent SARS-CoV-2 replication transcription complex
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The development of effective therapeutics against COVID19, caused by SARS-CoV-2, as well as preparedness for possible future coronavirus outbreaks, heavily relies on our mechanochemical knowledge of the viral genome processing machinery. SARS-CoV-2 expresses 16 non-structural proteins (nsp1-16), with most nsp's assembling into the Replication Transcription Complex (RTC), which efficiently synthesizes all viral RNAs in the host cell from the very long coronavirus genome (~30 kb). The core RTC consists of the nsp12-polymerase and the elongation co-factors nsp7 and nsp8 (1:1:2 stoichiometry), which are sufficient to ensure efficient synthesis. Despite the many structural studies already reported, the precise role of the co-factors and the mechanism of assembly of the core RTC remain unknown. Here, we use high-throughput magnetic tweezers to monitor the kinetics of assembly of the core RTC preceding primer-extension at the single molecule level in different nsp's conditions. Our data demonstrate that the assembly step largely dominates the overall RNA synthesis duration and that the nsp co-factors don't affect elongation dynamics, but rather speed up the assembly process. Using our data to build a mechanochemical model of assembly we reveal that a rate limiting barrier separates RNA bound complexes from becoming elongating core RTCs. Our findings support a model in which nsp8 recruits nsp12-polymerase at the primer template. We further propose that an RNA bound nsp12-nsp8 competes with nsp7 for the binding of another nsp8, where nsp7 increases the assembly rate by 2- to 3-fold. Our results clearly demonstrate that inhibition of the contacts between nsp7 and nsp12 or nsp8 could serve as an effective strategy to combat viral replication as a whole.

836-Plat
High-throughput, quantitative measurements reveal the biophysical mechanisms by which transcription factor mutations drive disease
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The Myc-Max-Mad triad of basic helix-loop-helix (bHLH) human transcription factors (TFs) regulate cell cycle progression. Mutations to or overexpression of these TFs drive aberrant expression of a large fraction of the genome in over 40% of cancers. Understanding the biophysical mechanisms by which alterations in these TF complexes—both the transcriptionally inactive Max-Max homodimer and the strongly activating Myc-Max heterodimer—disrupt function could therefore reveal new therapeutic approaches to restore normal gene expression and prevent proliferation.
Towards this goal, we applied a high-throughput microfluidic assay to quantify the effects of 235 Max mutations on DNA-binding affinity, DNA-binding specificity, and dimerization. We quantified the surprising and diverse mechanisms by which Max allelic variants can alter TF function to drive disease. Some variants alter specificity for non-canonical, low affinity motifs. Yet, other variants alter function by rendering the homodimeric Max unable to bind DNA, but are still functional in the disease-associated, Myc-containing heterodimer. Moreover, comparison of DNA specificity landscapes between Max and a related bHLH TF (with highly similar contacts but higher specificity) additionally reveal how subtle changes in amino acid sequence can dramatically impact promiscuity. Together, these data provide insights into how mutations perturb many facets of transcription factor binding — affinity, specificity, dimerization, and more — and paint a holistic picture of residue-level effects on complex TF function.

837-Plat
Molecular features underlying phase separation of protein-nucleic acid condensates revealed by a new coarse-grained DNA model
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Recent advances in residue-level coarse-grained (CG) computational models for proteins have enabled molecular-level insights into the properties of protein condensates. However, most of the existing CG nucleic acid models include anisotropic potentials between bases that are computationally demanding and are not fully compatible with the existing CG protein models. Here, we present a new CG DNA model that utilizes isotropic potentials between bases and can be used for investigating protein-DNA complexes and nucleosome arrays, thereby enabling a mechanistic understanding of how molecular information is propagated up at the genome level in a computationally tractable manner. To demonstrate the suitability of our model to facilitate large-scale simulations with a molecular resolution, we simulate nucleosomes (mono, di, etc.) to generate equilibrium conformational ensembles. The simulation data provide essential insights into the role of chromatin in the liquid-liquid phase separation of HP1α protein. We find an extensive network of interactions between HP1α, DNA, and flexible histone tails in the condensates formed by these different biomolecules. The findings of this work illuminate the complex molecular framework that contributes to heterochromatin regulation and function.

838-Plat
Multivalent interactions between RNA and RNA binding proteins drive differential intra-speckle positioning of RNA transcripts
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Nuclear speckles represent a type of membraneless organelles in higher eukaryotic cells which are rich in snRNP components, certain splicing factors,