simplicity of the dynamics, along with our use of biological inputs, is a significant step forward in understanding how chromatin dynamics affect both short-term (immediate response) and long-term (memory) cellular behavior.

1490-Plat
Multiplexing Cas9 activity with multi-target CRISPR

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We present multi-target CRISPR, an approach whereby a degenerate gRNA directs Cas9 to simultaneously target a large number of epigenetically diverse genomic locations. This multiplexing of CRISPR is enabled by targeting the highly abundant *Alu* repetitive elements. Combined with different next-generation sequencing readouts, multi-target CRISPR offered new insights into Cas9 activity and the ensuing cellular response. First, by interrogating the same protospacer in different genomic contexts, multi-target CRISPR showed that Cas9 binding is more efficient at open chromatin regions, and Cas9 cleavage is enhanced near transcribed regions. We then combined multi-target CRISPR with ATAC-Seq to unveil an increase in chromatin accessibility around Cas9 DSBs, which spreads over a window of $\sim$1 kb centered at the cut site. We attribute this chromatin opening to nucleosomes being evicted from the damaged region. Notably, the extent of chromatin opening is independent of the chromatin context in which the lesions occurs, suggesting that nucleosome eviction is a robust part of the DNA damage response. By combining multi-target CRISPR with photo-activatable gRNAs, we determined the characteristic time-scales of DSB-induced chromatin relaxation and found that nucleosome removal occurs within $\sim$30 min. Last, we combined multi-target CRISPR with a photo-deactivatable gRNA to measure chromatin dynamics after DSB repair completion. Once repair is completed, chromatin accessibility levels returned to their original pre-cleavage state within 1 h, revealing a quick repositioning of nucleosomes around the repaired cut site. Based on these measurements, we propose a model in which chromatin undergoes fast and transient opening in response to Cas9 breaks to facilitate recruitment of repair factors. In conclusion, multi-target CRISPR enables high-throughput studies of Cas9 activity and DNA repair *in cellulo*, revealing new insights on the interplay between Cas9 activity and chromatin.

1491-Plat
Isolating the effect of nucleosome modification driven phase separation on chromatin organization

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Several mechanisms regulate the three-dimensional organization of the genome. Though motor protein-driven loop extrusion has been studied extensively, the role of phase separation in establishing chromatin structure remains unclear. In this study, we aim to determine how histone modifications and variants affect nucleosome phase separation and drive chromatin organization in the absence of loop extrusion and other such mechanisms. Using Flory-Huggins theory and the mixing rule, we calculate the contact energy between nucleosome types from condensability scores obtained from *in vitro* condense-seq experiments. We run coarse-grained polymer simulations of chromatin with these contact energies and compare the resulting contact maps with experimental Hi-C maps of the same region. At the 10-20 Mb scale, simulations with contact energies inferred from polycation condense-seq recreate experimentally observed A/B compartment profiles. This suggests that heterochromatin formation at the compartment scale is regulated primarily by nucleosomal phase separation in the absence of other proteins. Future condense-seq experiments can be performed with various condensing agents besides polycations, including proteins such as HP1α. Our approach will provide insight into how these factors regulate chromatin architecture via phase separation. Disentangling the various mechanisms contributing to genome organization will enable us to more reliably predict and control gene expression, with applications in medicine and synthetic biology.

1492-Plat
Investigating human chromosome organization by whole-genome simulations

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Both theoreticians and experimentalists have been dedicating significant effort to studying chromosome organization and its relation to gene expression and regulation. Many experimental procedures were designed, such as Hi-C or ChIA-PET, to capture structural information of chromosomes. Several theoretical models were proposed to understand the principles underlying chromosome compartmentalization. MiChroM is a model that describes the compartmentalization behavior of interphase chromosomes. Compared to experimental data, the agreement of the *in silico* contact maps suggested that phase separation of A/B compartments is a key feature determining the structure of individual chromosomes. In this work, we use the Open-MiChroM software to simulate the 46 chromosomes of a GM12878 human cell at 50 kb resolution, seeking to understand whether the nuclear organization of the whole genome would rely on the same principles observed for the organization of a single chromosome. The ensemble of structures generated by our simulations captures chromosome organization within territories and phase separation of chromatin compartments. Inside a chromosome territory, active chromatin localizes preferentially in the periphery, while inactive chromatin lies on the inside. The intra-chromosomal contact maps and the shape metrics of each territory are consistent with experimental data. The resulting genome architecture resembles the inverted nucleus configuration, with active chromatin towards the periphery. In the interface between chromosomes, we also observe phase separation of compartments. However, the inter-chromosomal maps present higher contact probabilities for heterochromatin loci when compared to the experimental data. These results suggest that the interactions of chromatin with other nuclear features like the lamina greatly influences inter-chromosomal interactions while also affecting the overall positioning of chromosomes in the nucleus.

1493-Plat
Investigating the biophysical mechanisms regulating transcription in starved *Escherichia coli* via single-molecule tracking

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Bacteria have a robust stress response in starvation conditions, entering a quiescent stationary phase and remaining viable despite depletion of resources. An important component of this stress response is the upregulation of DNA-binding protein from starved cells (Dps) upon entry into the stationary phase. Dps promotes nucleoid condensation, protecting genetic information from harm. Intriguingly, this condensation does not affect the ability of RNA polymerase (RNAP) to access the nucleoid and transcribe RNA. The mechanism by which RNAP accesses the nucleoid under starvation conditions remains unclear. To investigate the dynamics of stationary-phase RNAP, we are applying live-cell single-molecule tracking of RNAP tagged with a photo-activatable fluorescent protein. In living *E. coli* cells, we find that compared to the dynamics of RNAP in the exponential growth phase, RNAP in the stationary phase spends less time bound to DNA, reflective of an expected decline in protein synthesis. Instead, stationary-phase RNAP spends the majority of its time in an intermediate dynamic state that we hypothesize results from frequent interactions with DNA as RNAP searches for its target sequence. Unexpectedly, the diffusion coefficient of this intermediate state increases in the stationary phase, even though the nucleoid is more compact and thus has a smaller pore size that could restrict motion. To isolate how Dps upregulation leads to changes in the dynamics of RNAP throughout the cell cycle, we over-expressed Dps and found that the RNAP dynamics were nearly identical to those measured with native Dps expression. Ultimately, these studies suggest that the interactions between RNAP and DNA are primarily biochemically driven, and do not depend on nucleoid pore size. Additionally, our results may indicate that Dps can be rapidly displaced by RNAP such that it does not interfere with target searching.

1494-Plat
Correlative visible light and soft X-ray tomography of *in-situ* mesoscale heterochromatin organization

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Recent evidence has revealed that cellular aging is accompanied by alterations in DNA methylation and histone modifications leading to a decrease in nucleosome packing density and heterochromatin remodeling. While these observations were obtained with multi-omics data (eg. Hi-C, ATAC-seq), the underlying ultrastructural mechanisms of mesoscale heterochromatin reorganization during cellular aging are still unclear. Towards this, we developed correlative light microscopy and cryo-soft X-ray tomography to analyze the alterations in heterochromatin organization in human mammary fibroblast cells.