PHASE SEPARATION

Crowd control

Cell https://doi.org/10.1016/j.cell.2018.05.042 (2018)

Credit: Dimitry Tegunov and Stefan Pfeffer

Measuring macromolecular crowding in cells relies on the use of tracer particles that convey the relative diffusion coefficient within a cell, but existing approaches perturb the cell. Delarue et al. developed fluorescently labeled scaffolding domains that self-assemble inside cells into stable particles of a defined size, which they termed genetically encoded multimeric nanoparticles (GEMs). Loss of mTORC1 signaling through rapamycin treatment or amino acid starvation increased the mobility of GEMs, which mutant screens and cryo-electron tomography linked to decreased ribosome concentration. The strong correlation between diffusion coefficient and ribosome concentration enabled the authors to develop a predictive model. In addition, higher ribosome concentration increased the prevalence of multivalent proteins entering a liquid droplet phase, whereas inhibition of mTORC1 decreased this phase transition. Overall, this study revealed new cellular mechanisms that regulate molecular diffusion and phase separation. *GM*

https://doi.org/10.1038/s41589-018-0109-1

NUCLEIC ACIDS

Caught in the act

eLife **7**, e36422 (2018)

Nonenzymatic RNA polymerization can occur through template-directed primer extension, in which nucleotide-5′-phosphoro-2-aminoimidazolides (2-AIpN) are suitable reactive monomers, and an imidazolium-bridged dinucleotide forms as an intermediate. Zhang et al. have now developed an approach for time-resolved crystallography studies on this system. The RNA primer–template complex was crystallized with nonreactive dGMP bound to the template-strand overhang of the RNA duplex. The crystals were then soaked with reactive 2-AIpG, which diffused into the large solvent channels and replaced the dGMP within minutes. Molecular snapshots of primer extension were obtained by freezing the crystals at different time points. First, two 2-AIpG molecules base paired with the two-nucleotide template overhang of the RNA duplex and formed the dinucleotide intermediate. Next, a phosphodiester bond formed between the primer 3′-hydroxyl group and the 5′-phosphate of the nucleotide in the +1 position. The dinucleotide binds in a pre-organized manner with a shorter distance to the primer than the monomer, explaining the higher reactivity of the primer for the dinucleotide. This method may also be useful for studying the role of metal ions during primer extension. *KK*

https://doi.org/10.1038/s41589-018-0110-8

CHROMATIN STRUCTURE

3D interaction hubs

Cell https://doi.org/10.1016/j.cell.2018.05.024 (2018)

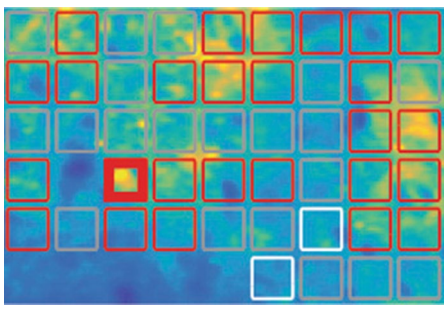
The intra- and interchromatin interactions in a cell provide a delicate layer of gene expression regulation. To detect multiple genome-wide and long-range DNA interactions occurring simultaneously within the nucleus, Quinodoz et al. developed the split-pool recognition of interactions by tag extension (SPRITE) method. SPRITE enables the interacting molecules within an individual complex to be uniquely barcoded and determined by sequencing and matching all reads with identical barcodes. Using this method, they quantitatively measured long genomic distance interactions and identified two major interchromosomal hubs arranged around nuclear bodies. By extending SPRITE to simultaneously measure DNA and RNA interactions, they identified an active hub with high gene density and active transcription around nuclear speckles, while the inactive hub was detected near the centromere in the vicinity of the nucleolus. The regional density of Pol II transcription rather than transcriptional activity of individual genes determined the proximity to nuclear bodies. Overall, this study provides a useful tool for characterizing the spatial organization of the global genome and further understanding of chromatin structure and gene expression regulation. *YS*

https://doi.org/10.1038/s41589-018-0112-6

NEUROBIOLOGY TOOLS

Dopamine gets lit

Science **360**, eaat4422 (2018)



Credit: AAAS

The ability to track the effects of neuromodulators such as dopamine (DA) on their target neuronal circuits is critical for understanding neuronal functions such as learning and motor control. To enable monitoring of DA signaling with high temporal and spatial resolution, Patriarchi et al. developed DA sensors consisting of a circularly permuted GFP inserted into inert human DA receptors, such that DA-induced conformational changes were coupled to GFP fluorescence. Optimizing the dynamic range and affinity of the sensors through mutation, the authors obtained dLight1.1 and dLight1.2. The sensors could monitor the concentrations of released DA in response to electrical stimuli and with receptor modulators such as cocaine, which blocks DA reuptake. As well, the sensors could report on DA signals associated with a spatially intermingled, task-specific DA transients map in the cortex, locomotion in dorsal striatum, and temporally sensitive DA transients in freely moving mice in learning and behavioral assays. Overall, these tools can be used to dissect the spatiotemporal coding of DA in learning, decision-making, and motor control, which occurs in milliseconds and at a cellular level. *MB*

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