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RESEARCH HIGHLIGHT

Engineering 3D genome organization

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The causal relationship between nuclear position and gene expression remains under intense debate. Qi and colleagues developed the CRISPR-GO system to efficiently reposition any genomic sequence to a specific nuclear compartment and examine its effect on transcription.

The nuclear positioning and three-dimensional (3D) organization of the eukaryotic genome, and its relationship with transcription regulation, DNA replication timing and genome stability are key unanswered questions in nuclear biology.[1] The analysis of nuclear architecture has intensified over the last decade due to advances in microscopy and chromatin conformation capture (3C)-based techniques. These approaches have been increasingly used as efficient tools to study the relationship between genome regulatory processes and its arrangement in the nucleus.[2] Interrogation of causal links between genome 3D structure and function has recently taken advantage of genome engineering methods including CRISPR to study the requirement of specific architectural proteins such as CTCF and YY1 in the regulation of interactions between enhancers and the expression of their target genes.[3] These CRISPR-based one-dimensional manipulations of specific genomic loci set the stage for experiments designed to reposition regions of the genome to specific nuclear neighborhoods in a recent landmark study by Wang et al.[4]

Eukaryotic genomes are partitioned into domains that segregate into two major compartments—compartment A comprising active genes and compartment B containing transcriptionally inactive sequences. Sequences in the B compartment correspond to lamina-associated domains (LADs) and are preferentially located in the nuclear periphery or nucleoli.[5] Activation of transcription concomitant with relocation from the periphery to the nuclear interior has been observed during lymphocyte development and neuronal differentiation.[6] To explore the causal link between the expression of a gene and its localization in the nuclear space, the LacO-LacI transgenic reporter system has been utilized, revealing that artificial tethering of regulatory loci to the nuclear periphery leads to gene repression.[7] However, this approach involves tedious multi-step screening for stable cell lines encompassing single LacO transgenic loci, preventing large-scale usage of this method. Wang et al.[4] now report a flexible chemical-inducible system for engineering interactions between specific regions of the genome and various nuclear compartments. This approach, named CRISPR-genome organizer (CRISPR-GO), allows investigators to analyze the functional consequence of reprogramming 3D interactions relative to specific nuclear compartments. Wang et al. employ the abscisic acid (ABA)-inducible ABI/PYL1 heterodimerization system[8] to make genome manipulation rapid, inducible and reversible (Fig. 1). Specifically, the ABI protein is fused with dCas9, which can be recruited to specific genomic loci by single-guide RNA (sgRNA). The PYL1 protein is then fused to a protein component of the specific nuclear compartment under study, such as the nuclear envelope (NE) protein Emerin, fused to GFP. The sgRNA-targeted genomic locus then recruits ABI-dCas9 but remains separate from the NE in the absence of ABA. Addition of ABA induces heterodimerization of ABI-dCas9 and PYL1-Emerin, leading to the relocation of the sgRNA-targeted locus to the NE (Fig. 1). CRISPR-GO exhibits ABA-induced tethering efficiencies of 47%-95% for a wide variety of genomic loci ranging from repetitive to single-copy sequences. Additionally, using hydroxyurea to arrest cells at the G1/S boundary, Wang et al. show that, although nuclear repositioning can take place in a fraction of cells in the absence of mitosis, complete repositioning to the nuclear periphery requires nuclear membrane breakdown in mitosis. To facilitate live cell imaging of the repositioning process in real time, dCas9 can be fused with an imaging component recognizable by a fluorescent dye in live cells. The ability to easily switch on or off genomic repositioning by adding or removing ABA makes CRISPR-GO an ideal tool for studying real-time dynamics of the process while taking advantage of the dCas9 live cell imaging technique.

Numerous membrane-less nuclear bodies (NBs) formed by liquid-liquid phase separation (LLPS) condensate proteins and nucleic acids in micron-scale nuclear compartments in eukaryotic cells and play important roles in the spatiotemporal regulation of gene expression and genome organization.[9] Among the reported NBs, Cajal bodies (CBs) are one of the best characterized and are involved in snRNA biogenesis and ribonucleoprotein assembly.[9] Wang et al. tethered transgenic LacO arrays or endogenous highly repetitive sequences to CBs using CRISPR-GO and then induced the formation of heterodimers between dCas9-ABI and PYL1-Coilin (a marker of CBs), which results in the localization of genomic loci of interest to CBs. Interestingly, time course recording of ABA addition plus dCas9-dye real-time live imaging allow the authors to capture a fast-rate formation and dissociation of CBs at regions of interest in a minute-scale time window. By fusing PYL1 with the PML protein, Wang et al. used CRISPR-GO to target genomic loci to PML bodies, which have been linked to chromatin remodeling in response to stress, tumorigenesis, and viral infection.[10] Similarly, by co-expressing ABI-dCas9 and PYL1-HP1α, the authors efficiently target regions of interest to heterochromatin.

To examine whether CRISPR-GO-mediated repositioning of genomic loci relative to various nuclear compartments influences transcription, Wang et al. targeted LacO transgenic arrays upstream of a Doxycycline (Dox)-inducible CFP reporter to the NE and observed a reduction in Dox-induced CFP reporter

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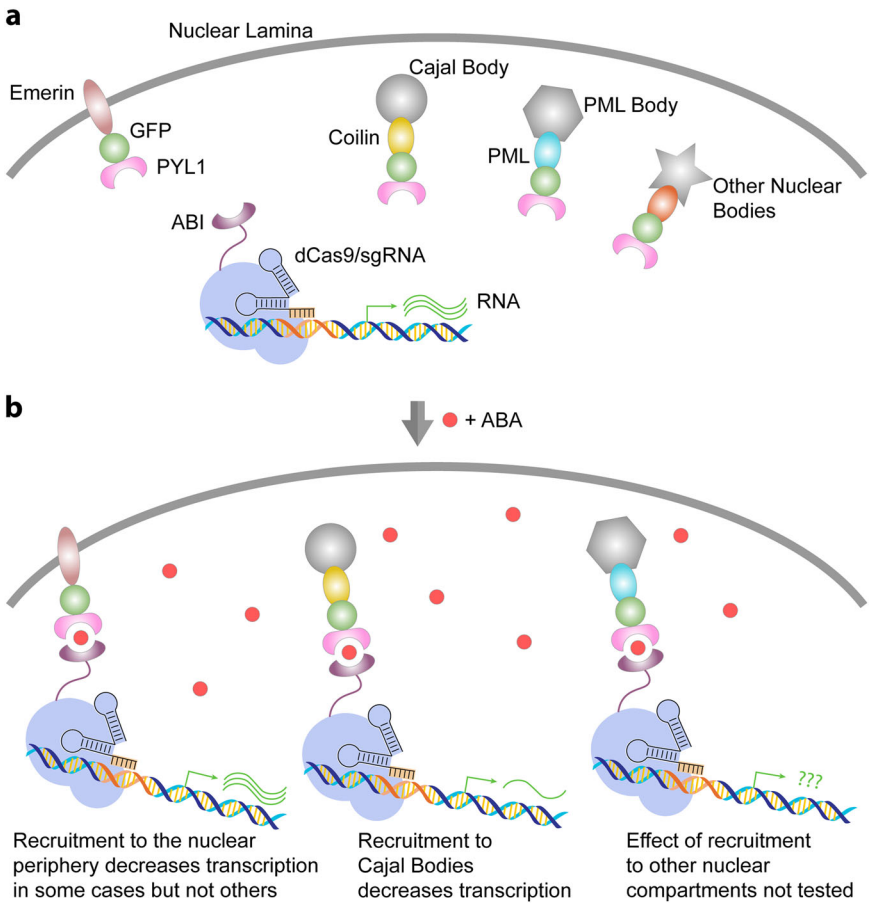

Fig. 1 Strategy of the CRISPR-GO technique. **a** GFP fused to PYL1 is targeted to a specific nuclear compartment via fusion with a protein present in the compartment under study, such as Emerin for targeting to the nuclear lamina. Subnuclear localization can be visualized by the fluorescence of GFP. Proteins specific for other compartments, such as Cajal or PML bodies, can be used for targeting to these compartments. At the same time, ABI is fused to dCas9, and the fusion protein is targeted to a specific region of the genome via appropriate sgRNAs. In the absence of ABA, dCas9 remains delocalized in the nucleus. **b** Treatment with ABA results in interactions between PYL1 and ABI, and subsequent recruitment of dCas9 and associated DNA to the corresponding nuclear compartment

expression. However, the repressive effects of the NE seem gene specific, since the transcription of other chr3 loci was not affected by their localization at the NE. In contrast, both LacO transgenes and endogenous loci are transcriptionally repressed when repositioned to CBs. Notably, CBs repress transcription of genes located up to several hundreds of kilobases away in linear distances from the sgRNA-targeted gene, possibly by 3D looping between the target loci and repressed genes. To test the functional outcome of genome repositioning during different stages of the cell cycle, Wang et al. targeted telomere sequences, which are normally dissociated from the NE at the G1/S phase boundary, to the NE during interphase and observed G0/G1 arrest followed by reduced cell viability. Targeting of telomeres to CBs promotes cell viability, perhaps due to the telomerase biogenesis function of CBs.[9]

The CRISPR-GO method developed by Qi and colleagues opens a new era in genome engineering by upgrading CRISPR-based genome editing from the linear genome to the 3D nuclear space. A long-standing question in the transcription field is the causal relationship between chromatin higher-order structure and regulatory function. CRISPR-GO will serve as a programmable and versatile tool for analyzing how changes in 3D genome organization affect transcription, genome stability and DNA replication. One question that remains is how CRISPR-GO overcomes stable interactions formed through LLPS to undergo genome reorganization, since the efficiency is variable among different genomic loci and target nuclear compartments.[4] Future studies should address the mechanisms by which the LLPS state driven by the local epigenetic and transcriptional status of loci in one compartment counteracts the tethering force of newly recruited chromatin-binding proteins into a different compartment. Another important question is whether the dynamic repositioning of chromatin to distinct nuclear compartments requires transition through the cell cycle, which may provide insights into the driving forces responsible for A/B compartment switches between different cellular states observed in Hi-C data. It will also be interesting to utilize CRISPR-GO to investigate the cellular function of other nuclear compartments/sub-compartments such as nucleoli and Polycomb bodies in a variety of biological processes.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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