



Minireview

Visualizing Live Chromatin Dynamics through CRISPR-Based Imaging Techniques

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The three-dimensional organization of chromatin and its time-dependent changes greatly affect virtually every cellular function, especially DNA replication, genome maintenance, transcription regulation, and cell differentiation. Sequencing-based techniques such as ChIP-seq, ATAC-seq, and Hi-C provide abundant information on how genomic elements are coupled with regulatory proteins and functionally organized into hierarchical domains through their interactions. However, visualizing the time-dependent changes of such organization in individual cells remains challenging. Recent developments of CRISPR systems for site-specific fluorescent labeling of genomic loci have provided promising strategies for visualizing chromatin dynamics in live cells. However, there are several limiting factors, including background signals, off-target binding of CRISPR, and rapid photobleaching of the fluorophores, requiring a large number of target-bound CRISPR complexes to reliably distinguish the target-specific foci from the background. Various modifications have been engineered into the CRISPR system to enhance the signal-to-background ratio and signal longevity to detect target foci more reliably and efficiently, and to reduce the required target size. In this review, we comprehensively compare the performances of recently developed CRISPR designs for improved visualization of genomic loci in terms of the reliability of target detection, the ability to detect small repeat loci, and the allowed time of live tracking. Longer observation of genomic loci allows the detailed identification of the dynamic characteristics of chromatin. The diffusion properties of chromatin found in recent studies are reviewed, which

provide suggestions for the underlying biological processes.

Keywords: chromatin dynamics, CRISPR engineering, genome imaging

INTRODUCTION

Chromatin is hierarchically organized in the nucleus to regulate gene expression and direct DNA replication and repair (Bickmore, 2013; Gibcus et al., 2013; Gilbert et al., 2010; Misteli, 2007; Rowley and Corces, 2018). Chromatin has also been shown to reorganize its three-dimensional (3D) structure to perform these functions (Agarwal and Miller, 2017; Chambeyron and Bickmore, 2004; Khanna et al., 2014; Seeber et al., 2018; Shaban et al., 2018; Shaban and Seeber, 2020). Thus, it is crucial to study how chromatin is spatially organized and how it changes structure over time. Several groups of methods have been developed to study the spatial organization of chromatin. A series of techniques based on deep sequencing of DNA following the ligation of closely located chromatin fibers, represented by the Hi-C technique, have successfully revealed the hierarchical domain structure in chromosomes (Fig. 1) (Lieberman-aiden et al., 2009). A full picture of small and large chromosomal domains, as well as how domain structure is regulated by proteins interacting with specific sequence motifs, has been revealed, even at the single-cell level (Nagano et al., 2013). Although the resolution of the Hi-C technique is only limited by sequencing

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depth and cost, it has fundamental limitations: (1) it does not directly provide real-space information, and (2) it shows only a snapshot of the domain structure in fixed cells at certain moments. However, it can be complemented by a technique based on direct imaging of genomic loci using target-specific probes, known as fluorescence *in situ* hybridization (FISH). This technique can be expanded by rigorously designing multiple sets of probes to uniquely target adjacent regions of the genome, which is known as the Oligopaint technique (Fig. 1) (Beliveau et al., 2012). A major limitation of FISH-based imaging methods is that they can only be applied to fixed cells because the target DNA must be denatured. Seeking DNA labeling systems that work in living cells, researchers engineered the CRISPR system to fluorescently tag specific genomic regions instead of editing them (Chen et al., 2013) (Fig. 1). This approach made it possible to study dynamic changes in chromatin structure and allowed temporal tracking of specific genomic regions, which had previously been possible only by inserting artificial protein-binding sequence motifs in the target region. However, there were some challenges in stably expressing the CRISPR system, efficiently delivering the system to target regions, labeling the target with high-density, and suppressing nonspecific background signals. Over the last decade, various modifications have been developed to address these issues. In this review, we highlight the technical developments that have revolutionized live imaging of the genome using CRISPR systems, and the dynamic behavior of chromatin revealed by CRISPR imaging.

TECHNIQUES TO VISUALIZE CHROMATIN STRUCTURE AND DYNAMICS

The spatial organization of chromatin has mainly been studied using chromosome conformation capture (Hi-C) techniques, which have been instrumental in elucidating the domain structure and interactions between distant domains (Farabella and Marti-Renom, 2020; Kim et al., 2019; Lieberman-aiden et al., 2009; You et al., 2021). Hi-C measurements revealed that chromosomes are organized into

two groups of topologically associated domains (TADs), compartments A and B. Compartment A has higher gene density and more activity in gene expression, compartment B has a lower gene density with a high density of H3K27me3 marks (Lieberman-aiden et al., 2009; Pope et al., 2014). Changes in genomic compartmentalization are associated with changes in transcriptional activity (Dixon et al., 2012). TAD structures typically form spatially insulated neighborhoods of approximately 500-1,000 kb in size. Consequently, *cis*-regulatory elements ('enhancers') can access gene promoters only within the same TAD and not across TAD boundaries (Dekker and Mirny, 2016; Dixon et al., 2016; Sexton and Cavalli, 2015). The TAD structure is largely invariant across cell types and appears to be highly conserved across species (Dixon et al., 2016). TAD boundaries are highly enriched with the insulator protein CCCTC-binding factor (CTCF) and the cohesin complex, which are strongly colocalized (Nora et al., 2017; Rao et al., 2017; Zuin et al., 2014).

These studies based on Hi-C measurements provide clarity on the hierarchical organization of chromatin with high genomic resolution. Single-cell Hi-C studies revealed high cell-to-cell variability in chromatin structure (Flyamer et al., 2017; Nagano et al., 2013; Ramani et al., 2017; Stevens et al., 2017), implying that the chromatin structure within each cell changes over time. Alterations in TAD structure have also been shown in cancer and senescent cells (Barutcu et al., 2015; Criscione et al., 2016; Taberlay et al., 2016). Genomic loci reposition themselves within the nucleus during embryonic development, and even during a single cell cycle when external stimuli affect gene expression (Chuang et al., 2006; Kohwi et al., 2013). However, the changing organization of chromatin over time and its biological implications have been explored in a relatively limited context, and the role of chromatin dynamics in regulating genomic functions is not fully understood. Studying chromatin dynamics in 3D nuclear space using live cell imaging can provide valuable information on how chromatin changes the spatial organization of genomic elements, controls enhancer-promoter interactions, repairs DNA damage, and regulates replication and tran-

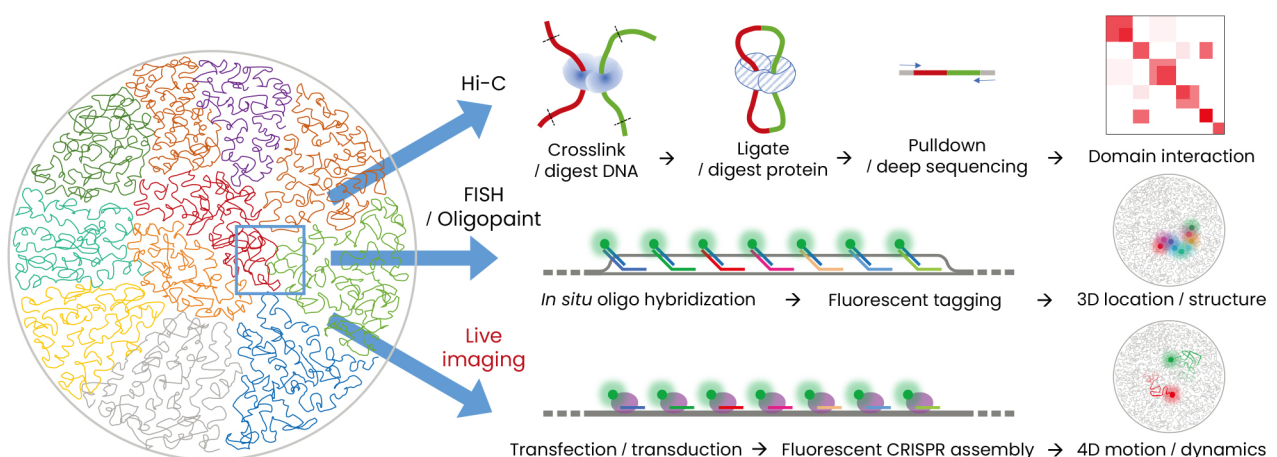


Fig. 1. Schematic procedures of various techniques to visualize chromatin structure and dynamics.

scription processes (Rowley and Corces, 2018). Revealing the structure-function relationship in the control of nuclear processes can help in understanding the relationship between the 3D organization of chromatin and its regulatory functions and can be utilized for therapeutic purposes in the diagnosis and treatment of genetic disorders such as cancer.

Early observations of chromosome territories were performed using DNA FISH (Cremer and Cremer, 2001; Lichter et al., 1988) and the dynamic repositioning of genomic loci during cell differentiation was reported later (Chambeyron and Bickmore, 2004; Clowney et al., 2012; Croft et al., 1999; Fraser et al., 2015; Küpper et al., 2007; Kurz et al., 1996; Mahy et al., 2002; Osborne et al., 2007; Volpi et al., 2000; Williams et al., 2002). While the Hi-C technique is mostly applied to ensembles of cells, FISH imaging explores chromatin structure in single cells and, combined with single-cell sequencing techniques, can potentially reveal the correlation between chromatin structure and gene expression control (Lee et al., 2021). The Oligopaint technique, an expanded version of FISH technique, was recently combined with the super-resolution microscopy technique to explore the 3D organization of megabase-sized chromatin regions with 30-kb resolution in individual cells, which found that the domain structure from Hi-C measurements matches that from real-space imaging remarkably well (Bintu et al., 2018).

Whole-genome sequence information and its functional status revealed by sequencing-based techniques such as ChIP-seq, ATAC-seq, and RNA-seq and high-resolution struc-

tural information from Hi-C and super-resolved chromatin imaging can be critically complemented by live-cell imaging approaches. Live tracking of single or multiple genomic loci allows assessment of the dynamic properties of chromatin by analyzing the time-dependent diffusion behaviors, temporal variation of the distance between distinct loci, and their correlated motions. Visualizing specific genomic loci in living cells requires a labeling system that can be expressed in the cell or injected into the cell and bind to the target site without denaturing DNA for alternative hybridization. It also requires the accumulation of probes at the site of interest in sufficient numbers to make the foci distinguishable from the background. In early studies of chromatin dynamics, genomic loci were visualized by integrating an array of *lac/tet* operator sequences (*lacO/tetO*) in the target locus to bind the *lac/tet* repressor protein fused with a fluorescent protein (Marshall et al., 1997; Michaelis et al., 1997; Robinett et al., 1996; Roukos et al., 2013). These systems required a ~10 kb repetitive array of operator sequences inserted into the target locus. To target unmodified genomic loci, transcription activator-like effectors (TALEs) were programmed to bind specific unaltered genomic loci, but the proteins had to be customized for each target sequence of interest.

More recently, the CRISPR technique, originally developed for genome editing, has been successfully applied to genome imaging. Highly specific binding to intact DNA containing the target sequence defined by the single guide RNA (sgRNA) makes it an ideal choice for live genome labeling. The origi-

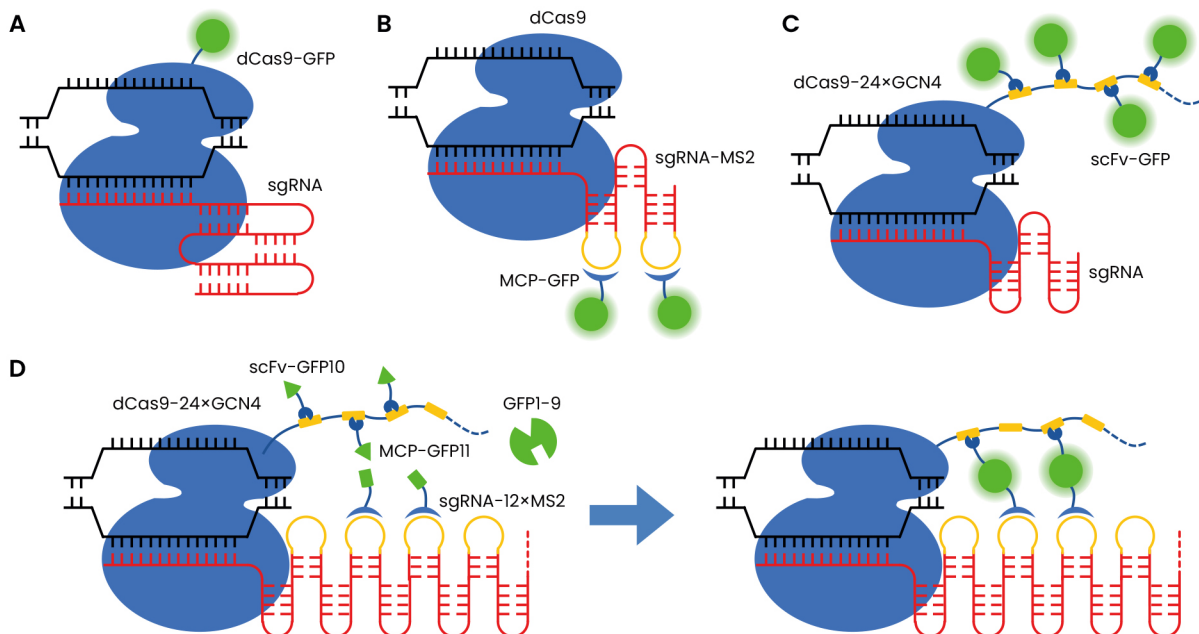


Fig. 2. Schematics of various CRISPR designs. (A) A CRISPR system with dCas9 fused to EGFP. (B) A CRISPR system with an sgRNA-MS2 scaffold recruiting fluorescent MCP-GFP. (C) A CRISPR-SunTag system recruiting multiple fluorophores at the tail of dCas9. (D) A CRISPR system integrating the SunTag system, MS2 scaffold, and tripartite GFP that allows the fast-exchanging assembly of full GFP at the target-bound CRISPR complex.

nal approach used a mutant Cas9 protein deprived of DNA cleavage activity, named deactivated Cas9 (dCas9), fused with a fluorescent protein, EGFP, to target genomic regions containing many repeats of sequence so that the expression of a single kind of sgRNA can effectively label the target regions (Fig. 2A) (Chen et al., 2013). The CRISPR/dCas9 system is highly flexible because the sequence of sgRNA only needs to be changed to target a different locus, which is a major advantage over the TALEs-based approach. This system was used to image repetitive motifs in telomeres. Imaging of the MUC4 loci by targeting a non-repetitive sequence with a set of sgRNAs was also demonstrated. Target-specific labeling of telomeres and MUC4 loci was confirmed by colocalized foci using CRISPR labeling and DNA FISH labeling. Another study used similar fusion proteins to target repetitive motifs in pericentromeres, centromeres, and telomeres in mouse embryonic stem cells (Anton et al., 2014). Genome imaging based on the CRISPR/dCas9 system was further extended to multi-locus imaging utilizing the diverse PAM sequences of orthologous CRISPR systems. Specifically, dCas9 from *Staphylococcus aureus* (dSaCas9) that recognizes a PAM sequence of 5'-NNGRRT-3' was used in combination with the widely used dCas9 from *Streptococcus pyogenes* (dSpCas9), which recognizes a PAM sequence of 5'-NGG-3', by fusing them with distinct fluorophores (Chen et al., 2016). A similar strategy combined dSpCas9 with other orthologous CRISPR systems from *Neisseria meningitidis* (dNmCas9) and *Streptococcus thermophilus* (dStCas9) (Ma et al., 2015).

DEVELOPMENT OF CRISPR DESIGNS FOR IMPROVED SIGNAL QUALITY

To achieve robust and long-term multi-color CRISPR imaging through signal amplification, several groups have tried to integrate protein-binding scaffolds into sgRNA to recruit multiple fluorescent proteins to the target loci. Repeats of protein-binding motifs were inserted into the sgRNA and the proteins recognizing these motifs were fused to fluorescent proteins (Fig. 2B) (Fu et al., 2016; Ma et al., 2016; Maass et al., 2018; Qin et al., 2017; Shao et al., 2016; Shechner et al., 2015). The RNA-binding proteins were recruited to the sgRNA scaffold, while dCas9 localized the sgRNA at the target site. The most commonly used RNA-binding protein is the MS2 coat protein (MCP), which is derived from the bacteriophage MS2 RNA virus, which has high affinity and specificity to the MS2 sequence (Larson et al., 2011; Wu et al., 2012). Other proteins used in CRISPR imaging were PP7 coat protein (PCP), Com, lambdaN, and Puf1, which bind to their respective target RNA motifs and are orthogonal to one another (Chaudhary et al., 2020; Ma et al., 2016; Maass et al., 2018). Such a design was shown to suppress background signals originating from the non-specific aggregation of fluorescently labeled dCas9 proteins. This approach has also been used for multi-locus imaging by incorporating distinct RNA aptamers such as MS2 and PP7 motifs into sgRNAs for different targets and fusing MCP and PCP with distinct fluorescent proteins (Ma et al., 2018; Wang et al., 2016). Another approach was to use a peptide array of GCN4 that recruits single-chain variable fragments (scFv) of the antibody, known as the SunTag

system (Fig. 2C) (Tanenbaum et al., 2014). scFv fused to superfolder GFP (sfGFP) was used to amplify the fluorescence signal from each CRISPR complex.

Fluorophore-fused dCas9 was used in the first demonstration of CRISPR-based genome imaging but nonspecific aggregation caused background issues. Thus, it required tight control of protein expression level to achieve a decent signal-to-background ratio. Several approaches have been proposed to address this issue. A bimolecular fluorescence complementation assay, in which the fluorescent protein Venus is split into two fragments and fused to dCas9 and MCP, which bind the MS2 motif added to the tail of sgRNA, greatly reduced background and non-specific signals compared to earlier designs (Hong et al., 2018). A similar approach was used to split sfGFP into three parts, which made the assembly/disassembly of the fluorophore much more frequent, and combined it with the SunTag amplification system and a long repetitive sgRNA scaffold, resulting in faster recovery of the fluorescence signal and a large reduction in background signal (Fig. 2D) (Chaudhary et al., 2020). The use of the small solubility-enhancing tag, GB1, was also essential for reducing unwanted aggregation of the protein components. The combination of tripartite split sfGFP, SunTag system, and 12 repeats of MS2 binding sites demonstrated long term tracking of small loci with as few as 13 repeats of CRISPR target, even using a conventional epi-fluorescence microscope. An alternative approach is to label and assemble dCas9 and sgRNA *in vitro* and target genomic loci in living cells as well as fixed cells and tissues, termed as CASFISH (Deng et al., 2015; Ishii et al., 2019). The above approaches, their choice of promoters/cell types, target loci, and transfection methods are summarized in Table 1.

Improvement of sgRNA design is crucial in the development of CRISPR designs for more robust and efficient assembly of dCas9-sgRNA complexes at specific targets. In the study by Chen et al. (2013), an A-U flip and hairpin extension in the sgRNA design resulted in two-fold increase in the number of detectable puncta and a five-fold increase in signal-to-background ratio. With this design, the non-repetitive MUC4 loci could be detected with as few as 26 sgRNAs by lentiviral cocktail transduction. Using the CRISPR-SunTag system resulted in a 19-fold signal increase in telomere imaging compared to dCas9-EGFP in HEK293 cells (Tanenbaum et al., 2014). A dual-color labeling approach inserting two copies of MS2 binding sites to the original sgRNA exhibited two-fold higher photorecovery compared to dCas9-EGFP due to the high exchange rate of MS2-MCP binding, as demonstrated by the long-term imaging of human telomeres and centromeres (Shao et al., 2016). Another dual-color labeling approach using the MS2-PP7 system allowed simultaneous labeling of major and minor satellite regions in murine 3T3 cells by inserting two copies of MS2 and PP7 binding sites in the sgRNA (Fu et al., 2016). This study also demonstrated the co-labeling of *Igh* and *Akap6* gene loci on mouse chromosome 12, which was confirmed using DNA FISH. A modified sgRNA with 16 copies of MS2 binding sites was used for long-term tracking of endogenous loci throughout the cell cycle (Qin et al., 2017). The robustness of this design was demonstrated by labeling non-repetitive loci with only four

Table 1. List of CRISPR/dCas9-based live genome imaging systems and designs

CRISPR design	Promoter/reporter	Cell type /species	Targets	Transfection method	Investigation	DNA FISH validation	Reference
dCas9-EGFP	pTRE3G/EGFP	RPE/h, UMUC3/h	Telomeres, MUC4, MUC1	Lentivirus induction, stable cell line	Chromatin dynamics	Yes	(Chen et al., 2013)
dCas9-EGFP	CAG/EGFP	J1 embryonic stem cells/m	Telomeres, major and minor satellites	Transient transfection, Lipofectamine 2000	Chromatin dynamics	Yes	(Anton et al., 2014)
dCas9-SunTag _{24x} , scFV-GCN4-GFP	dSV40/sfGFP	HEK293/h	Telomeres	Transient transfection, PEI	Chromatin dynamics	No	(Tanenbaum et al., 2014)
dCas9 variants (Sp, Nm, St1)	EF1 α , SSFV, EFs, CMV-TetO/BFP, GFP, RFP	U2OS/h, RPE-1/h	Telomeres, C9-1, C9-2, MUC4, C13-1, C13-2	Transient transfection, Lipofectamine 2000, Lipofectamine LTX	Multi-locus imaging	No	(Ma et al., 2015)
dCas9 variants (Sa, Sp)	pTRE3G/EGFP, mCherry	RPE/h	Telomeres, α -satellite, Ch1R, MUC4, 5SrdNA, Ch17RCh7R, Ch19R	Lentivirus induction, stable	Multi-locus imaging	No	(Chen et al., 2016)
dCas9-SunTag, scFV-GCN4-sfGFP, mNeonGreen	CAG/sfGFP, mNeonGreen	HEK293T/h	Telomeres, Ch5R, Ch14R	Transient transfection, Lipofectamine 3000	Chromatin dynamics, SBR	Yes	(Ye et al., 2017)
dCas9-EGFP, MS2, PP7	pTRE3G/EGFP, mCherry	MDA-MB231/h, HeLa/h	Telomeres, centromeres	Lentivirus induction, stable cell line	Chromatin dynamics, long-term imaging	No	(Shao et al., 2016)
dCas9, MS2, PP7, boxB	CMV-TetO/BFP, GFP, RFP	U2OS/h	Telomeres, Cx, C14, C7, C1, C13, & C3 repeats	Transient transfection, Lipofectamine 2000	Multi-locus imaging	No	(Ma et al., 2016)
dCas9, MS2, PP7	MSCV, UbC	3T3/m	Major & Minor satellites, Akap6, lgh	Lentivirus induction, stable cell line	Multi-locus imaging	Yes	(Fu et al., 2016)
dCas9, MS2, PP7	pTRE3G, SFFV/EGFP, mCherry	U2OS/h, HeLa/m	Telomeres, low and non-repetitive loci	Lentivirus induction, stable cell line	Chromatin dynamics & positioning	No	(Qin et al., 2017)
dCas9, MS2, PP7, Puf1	EF1 α , hUBC/mVenus, mCherry, IRFP670	RPE-1/h, mESCs/m	Telomeres, <i>FIRRE</i> , <i>XIST</i> , <i>Firre</i> , <i>Ype4</i>	Transient transfection, Lipofectamine 3000	Allele-specific imaging, nuclear positioning	No	(Maass et al., 2018)
dCas9-SunTag, MS2	CMV/split-GFPs, EGFP	HEK293AD, RPE-1	Telomeres, C9-1, X-114, low and non-repetitive loci	Transient transfection, Lipofectamine 2000	Chromatin dynamics, SBR	Yes	(Chaudhary et al., 2020)

h, human; m, mouse; PEI, polyethylenimine; SBR, signal-to-background ratio.

types of sgRNAs.

DYNAMIC BEHAVIORS OF CHROMATIN REVEALED BY CRISPR IMAGING

Live tracking of genomic loci for an extended period enables the precise analysis of their dynamic behaviors. Diffusion behaviors have been of particular interest because they reveal the physical characteristics of chromatin fibers and their local environment, such as compaction, spatial confinement, and dynamic rearrangement. Owing to the complex local environment, the diffusion behavior of chromatin differs from the Brownian motion of freely diffusing particles. It is better described by anomalous diffusion, meaning that the mean square displacement (MSD) of observed loci is fitted as $MSD = At^\alpha$ with α not equal to 1. A constrained motion exhibits α smaller than 1, and directional motion exhibits α larger than 1. Various factors affect chromatin dynamics, including the nuclear location of the loci, cell cycle, metabolic state, and DNA damage. Several studies have reported modulated or directional motions of chromatin upon the induction of local or global DNA damage, implying the role of chromatin reorganization in the DNA damage response, which have been discussed elsewhere (Agarwal and Miller, 2017; Seeber et al., 2018; Shaban and Seeber, 2020).

To assess the diffusion behaviors of chromatin by tracking single-particle trajectories, an early study used a *lacO* array to track nucleoplasmic, peripheral, and nucleolar loci and showed that the nucleolar or peripheral loci are significantly less mobile than the nucleoplasmic loci (Chubb et al., 2002). Another study using a similar labeling method and a novel particle-tracking method in a two-photon microscope revealed heterogeneous behavior of constrained diffusion and fast directional motions (Levi et al., 2005). The dynamics of telomeres were studied by tracking fluorescently tagged TRF1 or TRF2 proteins and mixed diffusive behaviors dependent on the observation time scale were observed (Bronstein et al., 2009), while another study reported a consistent subdiffusive behavior of telomeres (Cho et al., 2014). A later study revealed slow subdiffusion of telomeres that turned into fast normal diffusion due to the loss of lamin A proteins (Bronstein et al., 2015). From a different approach tracking fluorescently tagged individual H2B proteins, a subdiffusive behavior of nucleosomes that is dependent on the nuclear location and chromatin state has been reported (Shinkai et al., 2016).

CRISPR-based genome labeling enables the tracking of genomic loci without inserting exogenous DNA or being limited to genomic regions that possess exclusively binding proteins, such as telomeres. Using a CRISPR labeling system with a novel technique to package and deliver multiple sgRNAs, an enhancer region for the *Fgf5* promoter was shown to exhibit subdiffusive motion and increased mobility upon transcriptional activation (Gu et al., 2018). Using a multi-color CRISPR labeling system, the relative and centroid motions of genomic loci pairs situated kilobases to megabases apart on the same chromosome were measured, revealing that both local fluctuations and translational motions of the centroid changed in a cell-cycle-dependent manner (Ma et al., 2019). Using a

recently developed CRISPR labeling system that suppresses background signals, 3D motions of genomic loci ranging in size from kilobases to megabases were tracked to reveal mixed diffusive behaviors of subdiffusion to normal diffusion depending on the observation time scale (Chaudhary et al., 2020). Spatial motions of chromatin observed so far exhibit various types of diffusion behaviors with broadly distributed diffusion parameters. The imaging systems, target loci, temporal resolution, and cell lines used in the above studies and the diffusion parameters D and α found from these are summarized in Table 2.

PERSPECTIVES ON FUTURE GENOME IMAGING TECHNIQUES

Despite the innovations made by many research groups to revolutionize genome imaging using CRISPR systems, CRISPR-based approaches face several challenges for their application to genome-wide targets. First, targeting non-repetitive genomic loci remains challenging, as it requires not only packaging a large set of sgRNAs, but also stably expressing and assembling them with dCas9. To overcome these issues, new strategies have been developed to directly deliver *in vitro* transcribed sgRNA or recombinant ribonucleoprotein complex of dCas9-sgRNA, which also facilitates the construction of a multi-color labeling scheme (Geng and Pertsinidis, 2021; Wang et al., 2019). This approach allows the integration of a CRISPR imaging system with programmable switches, for example, by putting blockades on sgRNA that can be displaced by single-stranded DNAs as the switching input (Hao et al., 2020). Synthetic sgRNAs with terminal azide modifications have been shown to enable click chemistry, suggesting their use for site-specific, multiplexable chemical tagging of chromatin (George et al., 2020). Off-target binding of CRISPR complexes is another technical challenge in CRISPR-based imaging. The presence of off-target sites at high density may lead to false-positive locus detection, especially as it is attempted to decrease the size of the CRISPR array (Kuscu et al., 2014; Zhang et al., 2015). DNA FISH assays are typically used to confirm the target specificity of CRISPR labeling. However, as it requires strong denaturing conditions, it may not preserve the local chromatin structure and is not highly compatible with protein-based CRISPR labeling. Because the target binding efficiency and off-target effect of CRISPR complexes depend on the stability of sgRNA and the accessibility of target loci (Doench et al., 2014; Kuscu et al., 2014; Wang et al., 2014), a systematic protocol for target selection is needed for optimal application of CRISPR-based imaging to small, non-repetitive genomic regions.

A reliable CRISPR-based imaging system could reveal new insights into how cells regulate genomic functions by modulating chromatin structure and dynamics. As DNA repair, replication, and transcription involve active reorganization of chromatin, live visualization of chromatin motions will elucidate new functional mechanisms of these nuclear processes. Various chromatin-associated proteins may interfere with the functioning of CRISPR. For example, nucleosomes and nucleosome remodelers modulate the functions of CRISPR/Cas9 (Isaac et al., 2016). If chromatin structure affects CRIS-

Table 2. Diffusion behaviors of genomic loci measured in mammalian cells by single particle tracking

Labeling scheme	Target loci	Diffusion coefficient, D ($\mu\text{m}^2\text{s}^{-1}$)	Anomalous exponent, α	Diffusion mode	Temporal resolution	Cell lines	Reference
<i>lacO</i> array	5p14, 3q26.2 (nucleolar), 13q22, 13p, 1q11 (peripheral, nucleolar)	1.25×10^{-4}	-	Subdiffusion	60 s	HT-1080	(Chubb et al., 2002)
<i>lacO</i> array	Random	2.40×10^{-4} (slow) and 3.13×10^{-3} (fast)	-	Confined diffusion (short term) Normal diffusion (long term)	30 ms	CHO	(Levi et al., 2005)
TRF2-GFP	Telomeres	1.8×10^{-3} to 2.5×10^{-3}	0.32 (short term) to 1.15 (long term)	Subdiffusion to normal diffusion	10 ms	U2OS	(Bronstein et al., 2009)
TRF1-mCherry	Telomeres	3.3×10^{-2}	0.8	Subdiffusion	2 min	U2OS	(Cho et al., 2014)
TRF2-GFP, <i>lacO</i> array	Telomeres, centromeres, β -globin gene	4×10^{-4} to 3×10^{-3}	0.4 to 0.7	Subdiffusion	0.4 s	U2OS, HeLa, NIH3T3, MF, MEF	(Bronstein et al., 2015)
H2B-PA-mCherry	Single nucleosome	1.8×10^{-2} (interior) 1.3×10^{-2} (periphery)	0.44 (interior) 0.39 (periphery)	Subdiffusion	50 ms	HeLa	(Shinkai et al., 2016)
CRISPR-CARGO	<i>Fgf5</i> enhancer	3.5×10^{-3}	0.53	Subdiffusion	0.2 s	mESC	(Gu et al., 2018)
CRISPR-Sirius	Loci pairs on <i>chr19</i>	4.10×10^{-4} to 7.95×10^{-3} (relative) 4.35×10^{-4} to 4.25×10^{-3} (centroid)	0.107 to 0.512 (relative) 0.341 to 0.782 (centroid)	Subdiffusion	3 s	U2OS	(Ma et al., 2019)
CRISPR-Sun-Tag-SplitGFP	Telomere, C9-1 (9q12), FAM20C, HTT, X-114, X-76, C1-121, C9-78	5.0×10^{-5} to 1.0×10^{-3}	0.75 to 1.10	Subdiffusion to normal diffusion	6 s	AD293, RPE-1, IMR-90	(Chaudhary et al., 2020)

PR function, CRISPR labeling may also affect chromatin structure. The effect of CRISPR labeling on chromatin structure has not yet been fully addressed. Although dCas9 does not have endonuclease activity, the persistent occupancy of a genomic region by CRISPR complexes in large numbers would interfere with the native structure and dynamics of the region and could even induce DNA damage responses. One possible approach to clarify this is to use the CASFISH technique to verify the microstructure of chromatin domains observed by live CRISPR imaging. The CASFISH technique allows visualization of the preserved structure of chromatin by fixing the cells prior to the input of the CRISPR components and does not require DNA denaturation, unlike conventional FISH techniques. Despite recent progress, there is still a need to develop non-interfering chromatin imaging techniques in living cells or model organisms. With continuing improvements in CRISPR-based genome imaging techniques, we anticipate that this will reveal the implications of chromatin structure and dynamics in biological processes.

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AUTHOR CONTRIBUTIONS

N.C. and H.K. wrote the manuscript. J.K.I. and S.H.N. created the figures.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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