Chromatin Live Imaging with Genome Editing Techniques: Switching from Scissors to a Lamp

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Summary Chromatin live imaging integrated with programmable DNA binding proteins derived from genome editing methods opens up the era of spatio-temporal analyses of chromatin dynamics. The DNA binding proteins, including a transcription activator-like effector (TALE) or a nuclease-dead CRISPR-associated protein 9 (dCas9), can be freely designed to bind a specific DNA sequence. Instead of a nuclease for DNA cleavage, a fluorescent protein is fused to TALE or dCas9 for visualization of chromatin. They enable us to observe the endogenous genomic loci without the fixation of cells, the denaturation of DNA for hybridization and the insertion of exogenous DNA sequences into targeted loci. Using these live imaging systems dependent on the interaction between *cis* endogenous DNA sequence and *trans* DNA binding proteins, we can analyze chromatin dynamics in living cells. Multicolor chromatin imaging based on different dCas9 proteins will be a powerful cytogenetical technique instead of multicolor FISH and chromosome painting.

Key words Genome editing, TALE, dCas9, Chromatin dynamics, Live cell imaging.

Chromatin dynamics plays an important role in the regulation of gene expression during development and differentiation (Schneider and Grosschedl 2007, Sexton *et al.* 2007, Rosa *et al.* 2013, Pontvianne *et al.* 2013, Matsunaga *et al.* 2013, Feng *et al.* 2014). Fluorescence *in situ* hybridization (FISH) has been useful to detect DNA distribution in nuclei and on chromosomes (Hoshi *et al.* 2011, Shibata and Hizume 2011, Ebadi-Almas *et al.* 2012, Shirakawa *et al.* 2012, Suto *et al.* 2012, Hizume *et al.* 2013, Ikeda *et al.* 2013, Kuroki *et al.* 2013, Ruan *et al.* 2013, Yokomi *et al.* 2013, Suto *et al.* 2013, Lombello and Pinto-Maglio 2014, Abo-Zeid *et al.* 2015, Kantek *et al.* 2015, Shibata and Hizume 2015, Matsunaga 2016). However, FISH supplies only a snapshot of subnuclear dynamics because it requires the fixation of cells and DNA denaturation with high temperature for hybridization. To analyze chromatin dynamics, live imaging methods were developed for visualization of chromatins (Bystricky 2015). Various chromatin proteins including histones, condensins and a proliferating cell nuclear antigen can be fused with fluorescent proteins for chromatin visualization (Kanda *et al.* 1998, Kimura and Cook 2001, Boissard-Lorig *et al.* 2001, Gerlich *et al.* 2003, Fang and Spector 2005, Fujimoto *et al.* 2005, Lermontova *et al.* 2006, Higashi *et al.* 2007, Takata *et al.* 2007, Matsunaga and Umeda 2011, Nozaki

et al. 2014, Yokoyama *et al.* 2016). However, these protein-based chromatin visualization methods label whole or partial chromatins and are not available for the detection of specific genomic loci. The next approach was based on fluorescent repressor/operator systems, such as the *lacO/LacI* system (Robinett *et al.* 1996, Hirakawa *et al.* 2015, Hirakawa and Matsunaga 2016a). The fluorescent bacterial repressor fusion protein binds to the operator sequence inserted in the genome (Hirakawa and Matsunaga 2016b). Although twin dots on the homologous loci can be detected by fluorescent microscopy, the insertion of ~9kb of artificial tandem operator repeats into the host genome sometimes induces the accumulation of DNA methylation in the inserted site and influences chromatin dynamics (Jovtchev *et al.* 2011).

To reinforce the weakness in conventional methods, live imaging methods based on programmable DNA binding proteins derived from genome editing techniques were developed (Fig. 1). These methods do not require the insertion of exogenous DNA sequences but depend on the interaction between *cis* elements (endogenous DNA sequences) and *trans* regulatory proteins (DNA binding proteins). A first-generation programmable DNA binding protein, a zinc finger protein fused to GFP, was used for the visualization of repetitive DNA sequences (Lindhout *et al.* 2007). However, the zinc finger protein is limited in its target site selection because of context-dependent interactions with neighboring zinc fingers (Gaj *et al.* 2013).

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Fig. 1. Chromatin live imaging with programmable DNA binding proteins, TALE-FP, dCas-FP and dCas CP-FP.

To overcome the limitations and complexity in design of DNA binding specificity, the codes of nucleotide recognized by repeat variable diresidues (RVDs) have been established in a second-generation programmable DNA binding protein, a transcription activator-like effector (TALE)-binding site derived from plant pathogenic bacteria *Xanthomonas* spp. (Boch *et al.* 2009, Moscou and Bogdanove 2009). By combining an engineered TALE with fluorescent proteins (TALE-FP) (also known as TGV, TALE-color, TALE-light and dTALE), TALEs are used for the visualization of repetitive sequences including centromeres and telomeres in mammalian cultured cells (Miyanari *et al.* 2013, Ma *et al.* 2013, Thanisch *et al.* 2014, Miyanari 2016). This system was also used for the visualization of 359-bp repetitive sequences on the X chromosome and 10 bp-repetitive sequences in the pericentromeric region in *Drosophila melanogaster* (Yuan *et al.* 2014, Yuan and O'Farrell 2016). This system was also successful in the visualization of telomeric sequences, 180-bp centromeric sequences, and 18S and 5S rDNA sequences in *Arabidopsis thaliana* (Fig. 1) (Fujimoto *et al.* 2016).

Systems of a third generation programmable DNA binding protein, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), are also used for genome visualization (Chen *et al.* 2016a). A CRISPR/Cas9 system is a two-component system consisting of Cas9 from *Streptococcus pyogenes* and a single guide RNA (sgRNA), which directly interacts with the Cas9 nuclease at a specific locus in the genome (Jinek *et al.* 2012). Nuclease-dead Cas9 (dCas9) was fused with fluorescent proteins (dCas-FP) and co-introduced with sgRNAs for the target site (Fig. 1) (Chen *et al.* 2013, Anton *et al.* 2014, Lane *et al.* 2015). When multiple sgRNAs were introduced to the cells, even non-repetitive single locus could be visualized (Chen *et al.* 2013). Multicolor versions of dCas-FP were developed

using dCas from other bacterial orthologues (Ma *et al.* 2015, Chen *et al.* 2016b). Furthermore, to enable simultaneous visualization of multiple genomic regions with higher sensitivity, three-component systems were developed using dCas, sgRNA with stem loop motifs and the stem loop RNA binding coat proteins with fluorescent proteins (Fig. 1) (dCas CP-FP) (Ma *et al.* 2016, Shao *et al.* 2016, Wang *et al.* 2016, Fu *et al.* 2016). In this system, only one dCas is needed, and each fluorescent signal can be separated by the RNA stem loop motif—the coat protein pair. Moreover, a repeating peptide array, SunTag, which binds a single-chain fragment variable fused to GFP, was developed for signal amplification (Tanenbaum *et al.* 2014). Multiplex imaging with signal amplification will improve the ratio of signal to noise and enable us to analyze reliable chromatin dynamics on a single locus.

The relationship between chromatin mobility and double strand breaks (Lottersberger *et al.* 2015) or gene expression (Ochiai *et al.* 2015) was analyzed by these chromatin live imaging techniques. In the near future, chromatin live imaging with multicolor dCas-FP and multiple guide RNAs will allow simultaneous visualization of multiple specific chromosomes instead of multicolor FISH and chromosome painting. The advance in chromatin live imaging will contribute to produce new findings in DNA recombination, chromosome rearrangement, epigenetic regulation and nucleus organization.

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