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FISHing Out the Details of CRISPR Genome Tracks

Allison J. Coté[1] and Arjun Raj[1,*]
[1]Cell and Molecular Biology Graduate Group and Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania

The dynamic spatial organization of the genome affects gene expression control at many scales, from stem cell marker expression to enhancer-promoter pairs affecting a single gene (1). The lack of dynamic measurements in single cells, however, has made it challenging to determine whether structure of the genome is actually a cause of these changes in gene expression, or an effect of altered transcriptional states. In this issue, Guan et al. (2) report a method that provides a first step toward this technical goal by allowing researchers to begin to look at the movement of multiple pieces of the genome in real time in living nuclei.

The emergence of CRISPR/Cas9 has made it possible to target fluorescent labels to specific regions of DNA in live cells, but it has thus far had very limited multiplexing abilities (3–5). To visualize the interaction of several genomic regions, it will be necessary to simultaneously image the movement of those regions of DNA. Imaging multiple, unique smaller regions at once, however, remains a challenge. CRISPR/Cas9 DNA imaging is usually based on a Cas9-fluorescent protein fusion targeted toward a particular individual region of DNA based on the sequence of a coexpressed RNA that acts as a sequence-specific guide. However, this strategy does not easily translate to the ability to distinguish multiple genetic loci: while it is possible to target many regions of DNA by expressing many different guide RNAs, the different regions will be indistinguishable from one another because the same fluorescent protein will be targeted to all of them. Guan et al. (2) have cleverly surmounted this challenge by developing a method that combines the dynamic information from CRISPR/Cas9 imaging of repetitive DNA in live cells with new DNA FISH protocols that enable the unique identification of these same loci post facto, thus drastically increasing the multiplexing potential of live-cell CRISPR/Cas9 locus tracking (Fig. 1).

Guan et al. (2) employ a single color CRISPR/Cas9 system to target up to four unique genomic loci for live cell imaging, and pair with iterative DNA FISH after fixation to identify which loci are which. They simultaneously express CRISPR and Cas9 fused with a fluorescent protein (EGFP), and many different guide RNAs that target each of their four loci. They track those loci via live imaging, but, at that point, cannot determine the identity of each spot. After fixation, they perform several rounds of DNA FISH to retroactively determine the unique identity of each spot (Fig. 1). DNA FISH can identify individual loci in fixed cells, but is limited to the number of fluorescent channels available for imaging. With iterative DNA FISH, Guan et al. (2) can image FISH on one locus, strip off those probes, and then perform another hybridization and image a different locus with the same fluorescent signature. Traditionally this approach was limited by long hybridization times for DNA FISH, but their advance in speed, from days of hybridization to minutes, may open the door for massively multiplexed DNA FISH, similar to many techniques that have arisen in recent years for profiling transcriptomes in single cells using iterative RNA FISH (6–8).

The use of a single color to track multiple loci allows for other fluorescent imaging methods to report other cellular states that might be the cause or result of those genomic movements, such as gene expression changes or cell cycle state. As a proof-of-principle application of their method, Guan et al. (2) combine their CRISPR/Cas9 tracking and DNA FISH techniques with popular methods for cell cycle tracking to examine replication timing of individual genomic loci. This combination would have been impossible with previous multiloci tracking methods because they traditionally rely on using several different fluorescent proteins to uniquely identify different loci (Fig. 1), either through the use of multiple different Cas9-related proteins fused with different fluorescent proteins, or different fluorescent proteins that bind to different guide RNAs (3–5). Not only are these methods considerably more complex and difficult than the method of Guan et al. (2), but the use of multiple fluorescent proteins makes it difficult to use other fluorescent channels to track

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*Correspondence: arjunrajlab@gmail.com

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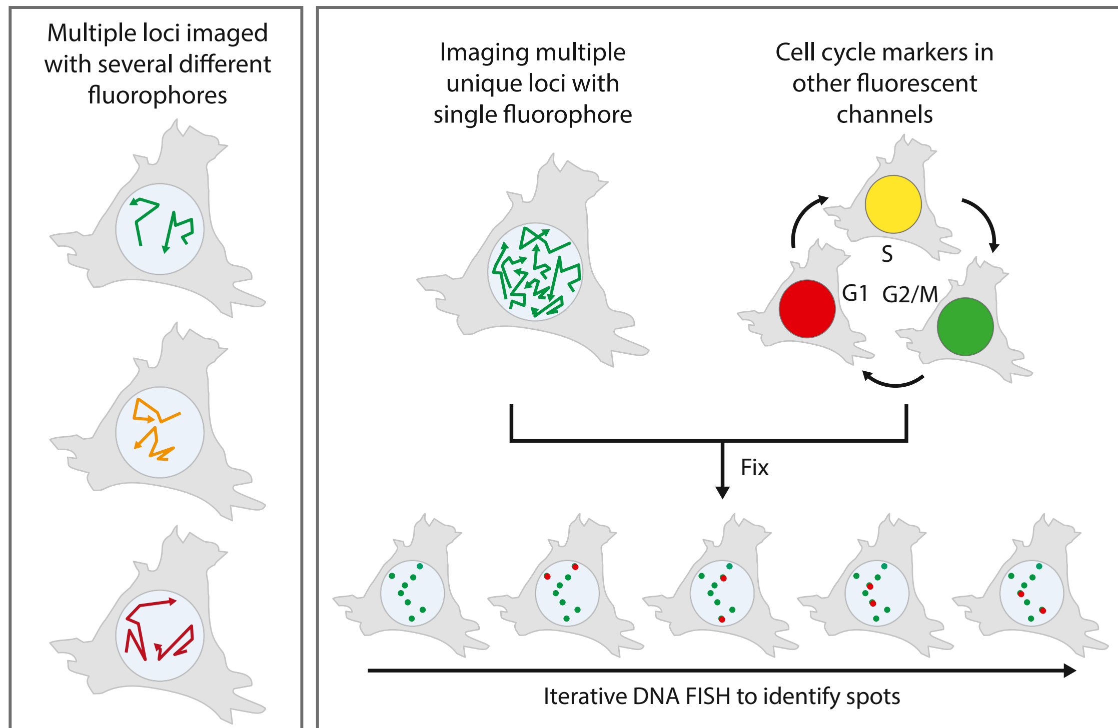

FIGURE 1 Schematic depicting traditional multiplexed live cell DNA tracking compared to live cell DNA tracking with iterative DNA FISH identification of spots demonstrated by Guan et al. (2). To see this figure in color, go online.

other cellular processes, such as—in this case—cell cycle. Although this combination will allow for imaging of multiple repetitive regions of DNA, targeting nonrepetitive regions of DNA using CRISPR/Cas9 remains a challenge, as does combining it with dynamic readouts of genome function. On the horizon, the method of Guan et al. (2) should combine nicely with advances in CRISPR/Cas9 imaging, such as imaging RNA or nonrepetitive DNA sequences (5,9), and provide a platform that dramatically increases the multiplexing ability of imaging genome movement. Together, these techniques help move us closer to a comprehensive picture of the effects of three-dimensional genome dynamics.

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