Three recent papers, published just weeks apart, describe the use of fluorescent TALEs to tag specific DNA sequences in live cells and, in one case, also in fixed cells, the latter with potential clinical applications.

When Walther Flemming described mitosis at the end of the 19th century, the live cell choreography of the chromosomes could be readily inferred, but the advent of phase-contrast microscopy and, later, polarized light microscopy gave cytologists of that era the thrills of their lifetimes, and this is so today as well. Indeed, I know not a single cell biologist, of any age, who does not rhapsodically marvel when seeing a cell divide, whether for the first or the 1000th time. Metaphase congression and anaphase movement are rarely equaled among the many dramatic phenomena of biology that can be conveyed in real-time. To be sure, everyone has other favorites, e.g., the beguiling differentiation pathway in Dictyostelium, cellularization of the insect embryo, and epiboly in vertebrate pre-gastrula embryos. Recent optogenetics-based video images of action potentials moving along live neurons are another spectacular show, and one can only imagine how much further this amazing technology will go in neurobiology. But while these and many other examples are all striking performances, they do not rise to the venerable status of iconic movies of mitosis or meiosis.

In contrast to observing their dynamic behavior during mitosis and meiosis, watching chromosomes move during interphase (and, to paraphrase Galileo, they do move) was a relatively neglected endeavor until the advent of GFP. The first study involved insertion of lac operators and expression of GFP-tagged lac repressors and revealed that the chromosomes of S. cerevisiae move in the interphase nucleus by constrained diffusion within confinement volumes, later confirmed in mammalian cells. Related studies specifically tagged telomeres in live cells either by exposure to a fluorescent oligo that hybridized to telomeres or inserting lac operators into the telomeric repeat or tagging telomeres by expressing GFP versions of telomere binding proteins.

Now, a new approach has been taken, based on transcription activator-like effectors (TALEs). In a flurry of just a few weeks, a boomlet of three papers appeared that each reported major findings and also, when taken together, signal the dawning of a new era in localizing DNA sequences in live cells.

Transcription activator-like effectors, TALEs, are distinctive types of transcription factors that were discovered in the plant pathogenic bacterial genus Xanthomonas. Their specific DNA sequence recognition domains consist of a tandemly repeating array of amino acids within which variations occurring predominantly at positions 12 and 13 confer remarkable differences in DNA sequence specificity, a property that has been dramatically exploited to design TALEs with extraordinary specificity for targets in a variety of genomes, typically in conjunction with a conjoined nuclease
to effect gene resection. However, it soon became apparent that TALEs lacking a nuclease but carrying a fluorescent tag would, in principle, be highly specific probes for DNA sequences, and it is this notion that drove the three recent studies. All three groups recognized, of course, that the initial proof of principle would need to involve repeated DNA sequences such as the telomeric repeat or centromere-associated satellite DNA sequences in order to maximize detection by the multiply bound TALE-FPs. The overall design principle of the TALE-FPs used in all three studies, with some minor variations, is shown for the case of telomeres in Figure 1. Note that TALE-FPs can be designed to bind either DNA strand. It is also to be emphasized that the key reason TALEs can be used to detect DNA sequences in live cells, as well as in fixed cells, is that they read the DNA sequence of one or the other DNA strand when interrogating it in double-stranded form.

In the first study, Miyanari et al. developed a method they termed TALE-mediated genome visualization (TGV) and used it to label peri-centromeric satellite DNA in mouse embryonic stem cells. Following expression of the TALE-GFP encoding plasmids after optimizing the TALE lengths for the DNA targets, they showed that the resulting signals in live cells impressively aligned with centromeres as defined by parallel fluorescent in situ hybridization in fixed cells and went on to track the intranuclear positions and spatial dynamics of these centromeric sequences throughout the cell cycle. These results were confirmed by chromatin immunoprecipitation of the peri-centromeric regions with GFP antibody. The authors then expanded the method to also tag telomeres and to also visualize centromeric DNA sequences in mouse embryonic stem cells and pre-implantation mouse embryos. Having characterized and optimized the method, Miyanari et al. then undertook an elegant generic application, using their TGV method to differentially tag either of two allelic telomeres in mouse F1 embryonic stem cells derived from an inter-specific parental cross in which the interrogated satellite DNA sequences diverged at two positions derived from an inter-specific parental. This was a remarkable finding, as the authors themselves comment, and speaks to the extraordinary DNA sequence recognition discrimination of TALEs.

Eight weeks after the Miyanari et al. paper appeared, Ma et al. reported the use of fluorescent TALEs to tag both telomeres and centromeres in live human osteosarcoma cells. They designed versions of these TALEs with a variety of fluorescent colors and also systematically optimized their lengths. After transfection of the appropriate plasmids, they observed bright signals corresponding to telomeres and centromeres, and by generating a stable cell line expressing a telomere-specific fluorescent TALE, they were able to image the intranuclear dynamics of telomeres in time-lapse videos. In additional experiments they designed fluorescent TALEs specific for centromeric satellite DNA sequences unique to either chromosome 15 or 18, two of the human chromosomes that bear the targeted regions by FISH as well as based with protein markers for these chromocenters. One of the longstanding research interests of this group has been to visualize centromeric DNA sequences in live cells and went on to track the intranuclear positions and spatial dynamics of these centromeric sequences throughout the cell cycle. These results were confirmed by chromatin immunoprecipitation of the peri-centromeric regions with GFP antibody. The authors then expanded the method to also tag telomeres and to also visualize centromeric DNA sequences in mouse embryonic stem cells and pre-implantation mouse embryos. Having characterized and optimized the method, Miyanari et al. then undertook an elegant generic application, using their TGV method to differentially tag either of two allelic telomeres in mouse F1 embryonic stem cells derived from an inter-specific parental cross in which the interrogated satellite DNA sequences diverged at two positions derived from an inter-specific parental. This was a remarkable finding, as the authors themselves comment, and speaks to the extraordinary DNA sequence recognition discrimination of TALEs.

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DNA replication, and they were able to use their fluorescence TALE-labeling method to follow these satellite sequences through S-phase and demonstrate, in live cells, the replication timing of these major satellite sequences and the 3-D intranuclear positions of these late-firing replicons, constituting a reassuring in vivo validation of many biochemical or fixed cell studies. In another instance of independent extension of this labeling method, and one not addressed by either of the other two groups, Thainisch et al. conducted fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) experiments to assess the intranuclear mobility of their GFP-TALE, being prompted to do so by their astute observation that the TALE was released from its chromosomal binding sites during mitosis. They observed a very large immobile fraction when areas around a chromocenter were bleached, compatible with relatively stable binding (during interphase), a conclusion that was reinforced by FLIP; in which large zones around a labeled site were bleached. Detailed analysis of these data and a comparison between GFP and TFP led to the conclusion that a very large fraction of the GFP-TALE is quite stably bound to its target sites, but nonetheless with a small fraction that displays a higher off-rate, this a very valuable parameter to know for continuing applications and refinements of TALE-FPs for DNA sequence visualization in live cells.

How do these new TALE-based methods compare with the previous ones? Although it would be foolish to think back to the early stage to claim an inherent superiority of the TALE-based methods, several points are nonetheless evident. The use of an oligonucleotide to tag telomeres has a cost of materials consideration greater than any plasmid-based method and also might be compromised in certain cells where the (passive or delivered) uptake and/or metabolic stability of the oligos is limiting. The method based on insertion of lac operators requires more plasmid construction and cloning than in the TALE-based methods, as well as the generation of stable cell lines. In addition, the tolemers at only one arm of each of only three chromosomes were tagged in that study, and one would ideally want complete coverage across the entire karyotype.

With the advent of this body of work using TALEs to localize and track DNA sequences, one might have predicted that it would only be a matter of time before the small guide RNAs (sgRNAs) of the CRISPR/Cas system might be similarly harnessed. It didn’t take long. On December 19, 2013, half-way between the Ma et al. (December 9) and Thainisch et al. (December 25) papers, a repeated DNA sequence localization study appeared based on the CRISPR/Cas system, and another CRISPR/Cas-based study localizing human chromosomal loci is imminent, adding to the momentum of these new approaches to the 21st century toolkit of chromosomal sequence visualization. With all this new, exciting progress, it is worthwhile to think back to the beginning, as epistemology. Everything in the half-century of research on transcriptional regulation since Jacob and Monod would have predicted that every single transcription factor would have to possess very high DNA sequence recognition specificity, although neither they nor anyone else could have anticipated the magnitude of this transcription factor diversity, the fact that these factors use cunning amino acid side-chain stereoisomerism to read the DNA sequence, and that there would ever be something like GFP. Nor could anyone have imagined that a bacterial genus that infects plants uses a refined type of transcription factors, TALEs, or that another amazing bacterial gene regulation pathway (CRISPR/Cas) was out there. Are there any better examples of how basic, unfettered research can lead to breakthrough progress? As we look forward to new applications of TALEs and CRISPR/Cas technology in genomics, let us encourage the reader to remember that, once again, the most fundamental biological research continues to bring biological research continues to bring forward to new applications of TALEs and CRISPR/Cas technology in genomics, and that the telomeres at only one arm of each of three chromosomes were tagged in that study, and one would ideally want complete coverage across the entire karyotype.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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