Corrections

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

The authors note that, due to a printer’s error, references 41–50 appeared incorrectly. The corrected references follow.


www.pnas.org/cgi/doi/10.1073/pnas.1323266111

DEVELOPMENTAL BIOLOGY

The authors note that, due to a printer’s error, references 25–29 appeared incorrectly. The corrected references are:


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PHYSICS

The authors note: “Our paper unfortunately missed reference to an earlier suggestion of the T6 structure (43). This work entitled ‘A hypothetical dense 3,4-connected carbon net and related B2C and CN2 nets built from 1,4-cyclohexadienoid units’ by M. J. Bucknum and R. Hoffmann was published in J Am Chem Soc 116:11456–11464 (1994), where the electronic structure of a hypothetical 3,4-connected tetragonal allotrope of carbon is discussed. The results in this article are consistent with what we find. The same group had also suggested a metallic carbon structure (44) that was published in J Am Chem Soc 105:4831–4832 (1983), which we also missed to cite. We thank Prof. Hoffmann for bringing these papers to our attention.”

The complete references appear below.


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CELL BIOLOGY

The authors note that, due to a printer’s error, references 25–29 appeared incorrectly. The corrected references are:


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Visualization of repetitive DNA sequences in human chromosomes with transcription activator-like effectors

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We describe a transcription activator-like effector (TALE)-based strategy, termed “TALEColor,” for labeling specific repetitive DNA sequences in human chromosomes. We designed TALEs for the human telomeric repeat and fused them with any of numerous fluorescent proteins (FPs). Expression of these TALE–telomere–FP fusion proteins in human osteosarcoma’s (U2OS) cells resulted in bright signals coincident with telomeres. We also designed TALEs for centromeric sequences unique to certain chromosomes, enabling us to localize specific human chromosomes in live cells. Meanwhile we generated TALE–FPs in vitro and used them as probes to detect telomeres in fixed cells. Using human cells with different average telomere lengths, we found that the TALEColor signals correlated positively with telomere length. In addition, suspension cells were followed by imaging flow cytometry to resolve cell populations with differing telomere lengths. These methods may have significant potential both for basic chromosome and genome research as well as in clinical applications.

Transcription activator-like effectors (TALEs) are able to recognize specific DNA sequences based on sequence composition of repeating oligopeptide elements (1). Advances in DNA cloning technologies have enabled facile assembly of TALEs for sequence-specific DNA recognitions as well as fusion of paired nucleases (TALENs) for genome engineering (2). Although TALEs and TALENs have rapidly become powerful tools for genome editing and transcription regulation (3), their intranuclear dynamics of DNA recognition are not well understood because they are typically directed to a single-copy sequence, thus limiting cytological studies and applications. It occurred to us that at least in cases of tandemly repeated DNA sequences, it should be possible to detect chromosomal sites of fluorescent TALE recognition and binding in live cells. By extension, we also considered it likely that fluorescent TALEs might be used as probes to detect DNA sequences in fixed cell preparations, as in conventional in situ hybridization but without a need to denature the DNA because TALEs read the target sequence in double-stranded form. Here we report the development of such methods as applied to both human telomeres and centromeric repetitive sequences.

Results

Our initial purpose in developing the methods to be reported stemmed from our interest in the relative intranuclear positions of telomeres and nucleoli in living cells. Our laboratory had previously developed methods to label and track ribosomal RNA out of nucleoli in living cells (4). The genes for ribosomal RNA lie close to telomeres in the short arms of several human chromosomes (5) and we pondered how we might label telomeres in live cells, as we had succeeded in doing for ribosomal RNA transcripts themselves. One of us (H.M.) considered that because TALEs recognize specific sequences in double-stranded DNA form, live cell applications would be feasible and that a telomere-specific TALE fused to a fluorescent protein might be a way to label the ends of chromosomes in live cells. As shown in Fig. 1F, TALEs were designed to recognize either DNA strand of the telomeric repeat. The TALE polypeptides were constructed as DNA plasmids with in-frame fusions to the desired fluorescent protein, followed by transfection and expression in human osteosarcoma’s (U2OS) cells. Fig. 1B shows the results of an experiment in which the TALEs TeII20 or TeIR20 targeting to either strand of the telomere repeats were coexpressed for 24 h. Numerous discrete fluorescent foci were observed in interphase cells with either of the two TALEs. TALEs recognize specific DNA sequences in native double-stranded DNA by reading from the major groove. The fact that coexpression of TALE–fluorescent proteins (FPs) designed for either strand of the telomeric repeat resulted in similar patterns of discrete nuclear foci with the two colors displaying complete spatial coincidence indicates that both strands of the telomeric repeat are accessible. U2OS cells are aneuploid, with ~65 chromosomes (6), and so are expected to have ~130 telomeres in G1 cells and ~260 in G2 cells. The number of TALE-labeled foci observed was typically less than 50 per nucleus, indicating either that not all telomeres were being detected or that many labeled sites are out of the focal plane. When serial optical sections were obtained by confocal microscopy (Fig. S1), the total number of labeled foci throughout the nucleus was between 50 and 70. It is of interest that in a previous study in which telomeres in live U2OS cells were labeled with a fluorescent peptide nucleic acid (PNA) probe (7) the number of interphase foci was also less than 70, indicating that a similar sized subpopulation of telomeres is available to the PNA and TALE probes, notwithstanding how chemically distinct their modes of DNA binding are. It is also noteworthy that there was considerable variation in the size/intensity of the TALE-labeled foci, suggesting either that certain telomeres are very heavily labeled or, alternatively, that some telomeres are clustered. To conduct time-lapse imaging we generated a stable cell line expressing the TeIIR20-mCherry and tracked dynamic movements of the foci during cell cycle progression.

Significance

Repetitive DNA sequences such as telomeres and centromeres are, like the chromosomes in which they reside, highly dynamic within the interphase nucleus, moving about by diffusion. Live cell imaging of these specific chromosomal sites has been limited and the approach presented in this study, based on expressed transcription activator-like effector (TALE)-fluorescent proteins, offers new opportunities in basic research on chromosome dynamics. In parallel, the described use of fluorescent TALEs as probes with fixed cells presents advantages over fluorescent in situ hybridization and may find particular applications in clinical and diagnostic settings.

Author contributions: H.M. and T.P. designed research; H.M. and P.R.-G. performed research; H.M. and P.R.-G. contributed new reagents/analytic tools; H.M. and P.R.-G. analyzed data; and H.M. and T.P. wrote the paper.

Conflict of interest statement: H.M. is an inventor on a US patent application filed by the University of Massachusetts.

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conventional FISH and thus might offer a shorter turnaround time. We constructed plasmids for coupled in vitro transcription–translation of telomere-specific TALEs fused in-frame with various fluorescent proteins, e.g., TALEGreen-TelR15 (Fig. 3A). When this was used as a probe with fixed U2OS cells, a pattern of discrete fluorescent foci were observed in interphase and also mitosis (Fig. 3B). To confirm that these signals represent binding of the TALE to telomeres, we carried out immunostaining for the telomere-specific protein TRF2 (15), which revealed colocalization with the TALE signals in both interphase (Fig. 3C) and mitotic cells (Fig. S4). As with the live cell experiments, the number of labeled foci in fixed cells was considerably less than the karyotypically predicted number of telomeres. Serial optical sections acquired by confocal microscopy of the fixed cells (Fig. S5) again revealed from 50 to 70 labeled foci, indicating that methanol fixation did not appreciably change the number of telomeres available to the TALE–FP probe. We also investigated the fixed cell method with HeLa cells, which have a similar degree of aneuploidy as U2OS cells, and again observed numerous discrete fluorescent foci in interphase and also mitosis (Fig. S6).

To determine how wide an array of FPs might be applicable to this method, we constructed a number of additional telomere–TALE–FP plasmids for coupled in vitro transcription–translation, as well as a telomere-specific TALE lacking a fused fluorescent protein but into which a fluorescent amino acid (green lysine) was incorporated during the translation step. As shown in Fig. 4, the entire spectrum of fluorescent proteins tested (Fig. 4A), as well as the green lysine–labeled TALE (Fig. 4B), resulted in comparable signals with the same spatial patterns as established in the live cell experiments. This indicates that, in this fixed cell version of the method, none of the fluorescent proteins, linked at a TALE’s C terminus, interferes with the TALE’s DNA sequence recognition nor is the fluorescence intensity problematically attenuated by intramolecular folding interactions back into the TALE. Moreover, the fact that the telomere TALE with an internally incorporated fluorescent amino acid (Fig. 4B) also gave the same pattern and with strong signal intensity demonstrates that chemical modification within the TALE polypeptide can be accommodated.

![Fig. 1](image1.png) **Fig. 1.** Illustration of telomere detection by TALEColor. (A) TALEYellow and TALERed probes were designed to target either strand of the telomere repeat by fusion of Venus or mCherry at C terminus. (B) U2OS cells were cotransfected with TALEYellow-TelR20 (Center Left) and TALERed-TelR20 (Center Right) and labeling was assessed in the live cells 24 h later. The Far Left panel is the phase-contrast images and the Far Right panel is the two-color overlays, respectively. (Scale bar, 5 μm.)

![Fig. 2](image2.png) **Fig. 2.** Live cell imaging of centromeres and telomeres by TALEColor. U2OS cells were cotransfected for 24 h with TALEmCherry-TelR20 to label telomeres together with one of three TALEs designed to recognize centromeric repeats. (Top row) TALEVenus-PanCen, a TALE predicted to bind all human centromeres. (Middle row) TALEVenus-Cen18, specific for an α-satellite higher order repeat on chromosome 18 (D18Z1). (Bottom row) TALEVenus-Cen15, a specific α-satellite higher order repeat on chromosome 15 (D15Z3). Overlay images are shown in the Far Right column. (Scale bar, 5 μm.)
Although live cell labeling of telomeres (Fig. 1) offers unique opportunities in basic cell biology and chromosome research, which we are pursuing, we wanted to explore the fixed cell method further. In particular, we asked how this TALE-based, fixed cell telomere detection method could be applied to human cell lines with differing telomere lengths, both to assess the interphase patterns of telomeres in these various cell lines and to also get an initial impression of whether or not the intensity of each focal fluorescent signal might be related to the telomere length. As mentioned earlier, we had so far no sense of whether we were labeling telomeres at some low, statistical level or some higher degree of labeling across the telomeric repeat possibly approaching or even reaching target saturation by the TALE. Fig. 5 shows the results of applying a TALE–telomere probe to fixed human cells having different average telomere lengths. U2OS cells have a wide array of telomere lengths, from <3 kb to >50 kb due in part to the operation of the alternative lengthening of telomeres (ALT) pathway (8). The HeLa cell line 1.3 has average telomere length ~23 kb (16). In contrast, weaker signals were observed in three other human cell lines known to have shorter telomeres: HeLa S3 (telomere length 2–10 kb) (17), Institute for Medical Research 90 (IMR90) (average length ~7.5 kb) (18) and retina pigmented epithelium 1 (RPE1) (~2–12 kb) (19), suggesting that under the constant probe conditions used in these fixed cell experiments, the signals obtained correlate with average telomere length to at least some degree.

To further explore the relationship between the TALE probe signal intensity and the length of telomeres, we set up a proof-of-principle experiment. The 1.3 and S3 HeLa cell lines were used as samples with longer vs. shorter telomeres (average length ~23 kb and 2–10 kb, respectively). The two cell lines were cocultured on coverglasses and subjected to TALE labeling (Fig. 6A). The telomere signals in HeLa 1.3 were much brighter as can be seen in separate or cocultured cells (Fig. 6A). Imaging flow cytometry was then used to analyze telomere length by TALE labeling of suspension cultures of the two cell lines. DAPI (a blue fluorescent DNA dye) and DRAQ5 (a far-red fluorescent DNA dye) were used to stain the DNA of HeLa 1.3 and S3, respectively, the cells were then mixed and TALE labeled with TALEGreen-TelR15, followed by FACS with the instrument’s parallel single cell imaging capability (Materials and Methods). As can be seen in Fig. 6B, Top row, Far Left, the two cell populations were clearly resolved on the basis of their two DNA labels, as expected, with each population displaying a typical cell cycle distribution including G1, S, and G2/M phases by DNA contents (Fig. 6B, Center Left in Top row and Center Left in Middle row). The TALEGreen-TelR15 signals were separated into three populations (Fig. 6B, Bottom row, Far Left). As can be seen in the overlay plots in the Center Left in the Bottom row of Fig. 6B, two populations having high and moderate telomere labeling were DAPI positive (HeLa 1.3), whereas a third population having low telomere labeling was DRAQ5 positive (HeLa S3 cells), compatible with the known telomere lengths of these two cell lines and consistent with the imaging from the coverglass cultures (Fig. 6A).

We next analyzed the various telomere labeling populations in each of the two cell lines with respect to the cell cycle (Fig. 6B). We gated the DAPI-positive cells as “R1” (high telomere labeling, shown in light green) and “R2” (moderate telomere labeling, shown as dark green). Meanwhile we gated the DRAQ5-positive population as “R3” (low telomere labeling, shown as teal). The individual or overlay plots of R1, R2, and R3 are shown in the Center Right column of Fig. 6B. It can be seen that the high telomere labeling population was typified by a greater proportion of S/G2/M phase cells, whereas the moderate telomere labeled population was enriched in G1 phase cells (Top row, Far Right).

The imaging flow cytometer allows us to image each single cell represented in the above plots. Representative images of cells in the R1, R2, and R3 populations (Fig. 6B) are shown in Fig. 6C. As can be seen in the Center Left four columns, the R1 population cells were DAPI positive (purple, representing HeLa 1.3 cells) and displayed high TALEGreen-TelR15 signals (green). The single-cell imaging revealed that the majority of this population consisted of mitotic cells. The R2 cell population (Center Right four columns) were also DAPI positive (and thus were HeLa 1.3) and had a moderate telomere labeling. This population was mostly G1 cells. Meanwhile the R3 cell population (Far Right four columns in Fig. 6C), defined as DRAQ5 positive (red, thus representing HeLa S3 cells) displayed low telomere labeling and consisted of all cell cycle stages.

Discussion

The methods we have developed and reported here seize upon the extraordinarily specific nucleotide sequence recognition capacity of TALEs and exploit, in particular, their unique affinity for targets in their DNA double-stranded form. We reasoned that given such sequence specificity and their preferential recognition of targeted sequences in native DNA, the attachment of a fluorescent protein to a given TALE would produce strong signals if the targeted sequence were tandemly repeated in the genome, and if the tethered fluorescent protein did not interfere with DNA sequence recognition in the TALE backbone. These hypotheses were borne out in the reported study. We were able to label the human telomeric repeats, the centromere sequence common to all chromosomes, and two centromere repeats specific to two chromosomes. In principle, it should be possible to extend this method to any other tandemly repeated DNA sequence element in a genome, such as the genes for ribosomal RNA (known to undergo expansion or attrition) or ones implicated in human diseases before and after genomic expansion. For example, we are now applying these methods to trinucleotide

Fig. 3. TALE–FPs label telomeres in fixed cells. (A) Diagram of TALEGreen-TelR15. (B) U2OS cells were fixed in 90% methanol and incubated with the probe. Shown are representative images in an interphase and anaphase cell. (C) After exposing fixed cells to the TALEGreen telomere probe, immunostaining was carried out with a TRF-2 antibody followed by a TRITC-labeled secondary antibody. (Top row) Probe imaged in both the green and red channels. (Middle row) TRF2 immunostaining imaged in both channels. (Bottom row) Probe and TRF2 immunostaining imaged in each channel. The Far Left column shows phase-contrast images and the Far Right column shows images in which both the probe and TRF2 merged onto DAPI staining. (Scale bars in A–C, 5 μm.)
repeat expansion diseases (20). The ability of this method to label specific human chromosomes also offers unique opportunities to detect aberrant chromosomes, and we are currently labeling and tracking the intranuclear dynamics of all three copies of chromosome 21 in human trisomy 21 patient cells (21) in relation to the territories they explore in these live cell studies. Given the extreme sequence specificity of TALEs (22), one might ask how far this method can be pushed. Obviously this will depend on the brightness of the fluor attached to a TALE and the genomic prevalence of the targeted DNA sequences (down to possibly single-copy genes). We can recall that the first demonstration of in situ nucleic acid hybridization involved repeated DNA sequences (the ribosomal RNA genes) (23) and that the method’s refinement to detect single-copy DNA sequences took some years. Another point to be emphasized is that the TALE-based method reported here docks a protein (the TALE) and its attached fluorescent protein onto a DNA sequence, so this is certainly a “cargo” as regards the live cell application of this method and this point must be borne in mind when interpreting the telomere and centromere dynamics observed.

Three previous studies have tracked telomeres in live human cells. In one, a lactose operator array was inserted into the telomeric repeat and was detected by expressing a GFP lactose repressor (8). In contrast, the method reported here does not involve a disruption of the telomeric repeat. A second study used a telomere-specific PNA probe (7), whereas another was based on GFP-tagged TRF1 or TRF2 (9). The live cell version of the presently reported methods, as applied to telomeres, is not claimed to be superior to these previous ones except for the avoidance of sequence interruption in the lac method. The present method does obviate the purchase of PNAs or the need for cloning to insert the lac repressor repeats.

The fixed cell variation of our TALE-based method has a number of key virtues. The preparation of fluorescent TALEs by coupled in vitro transcription–translation (Materials and Methods) is very time efficient compared with the synthesis or commercial procurement of fluorescent oligonucleotide probes for conventional FISH. Even more important is the very fast timescale of the TALE-based protocol. Starting with a coverglass culture, the steps of methanol fixation, rinse, probe incubation, and rinse takes less than 1 h compared with many hours in typical FISH methods. As H.M. anticipated when first envisioning this method, the ability of TALEs to recognize targeted sequences in double-stranded DNA obviates the need for a DNA denaturation step and we have also found that nonbound TALEs require only a single, rapid wash for removal.

Our initial studies with human cells with differing telomere lengths have hinted that the TALE-based signals may be reporting on average telomere length. Without a direct determination of how many TALEs bind along the telomeric repeat in any of the human cells we studied, we cannot presently assert that the method is reporting on telomere length in a truly quantitative way (i.e., with a linear relationship between telomere length and signal intensity over a wide range). This notwithstanding, it is clear that the signal intensities do correlate with the average telomere lengths of the cell lines. This result suggests that this method, with refinement, could have clinical applications in diagnostic situations where the average telomere length of a cell biopsy is relevant. A precise analysis of telomere length involves a molecular biology assay (24) but many hospital laboratories do not have the capability to carry out such techniques. In contrast, many clinical laboratories do have personnel familiar with in situ nucleic acid hybridization and our method is really a foreboding version of this. It seems plausible to envision that the TALE-based method reported here could be applied to a biopsy and reported back to the operating room within minutes, assuming that such a preliminary assessment of telomere length in these excised cells would have value in the subsequent surgery or patient treatment. Further refinements of the methods reported here may advance both basic research in human genomics and clinical applications.

Just after this manuscript was submitted, a study appeared in which TALE–FPs were used to label repeated sequences in both cultured mouse cells and embryos (25). The applications presented in that important study and the present one are different but complementary.

Materials and Methods

Construction of TALEColor Plasmids. TALEs for TALEColor were assembled using the TAL effector toolbox (26) obtained from Addgene. The designation vector for mammalian cell expression was derived from pcDNA4-Tohygromycin (27) and contains a FLAG tag, the simian virus 40 nuclear localization signal (NLS), and a truncated wild-type TALE backbone from the toolbox. For the specific telomere and centromere DNA probes used in this investigation, tandem repeats of 34-amino acid (aa) TALE monomers targeting

![Fig. 4. Spectral variants of TALEColor probes. (A) TALE-TelR15 probes were designed with various fused fluorescent proteins as indicated and applied to fixed U2OS cells. Images were captured in the appropriate channels (Lower row). (B) TALE-TelR15 probe with no fused fluorescent protein was produced carrying internal lysine residues labeled with a green dye (Materials and Methods). The labeling obtained (Upper row) was imaged and compared with that with the same TALE carrying fused mCherry (Lower row), with the Far Right column representing the respective images overlaid onto DAPI images. (Scale bar in A and B, 5 μm.)](image)

![Fig. 5. Telomeres compared by TALEColor in variety of human cell lines. U2OS, HeLa 1.3, HeLa S3, IMR90, and RPE1 cells were fixed and incubated with TALEGreen-TelR15 (Middle row). All of the images of TALEGreen-TelR15 (Middle row) are scaled to the same. The phase images are shown in Top row and images merged with DAPI are shown in the Bottom row. (Scale bar, 5 μm.)](image)
6–20 bp in the case of telomeric repeats and 20 bp in the case of the centromeric repeats were inserted into the destination vector to generate pTH-TelR15-mVenus and pTH-TelR15-mCherry for telomeric and pTH-PanCen-mVenus, pTH-Cen18-mVenus, and pTH-Cen15-mVenus for centromeres. To produce TALEColors by in vitro coupled transcription–translation for the fixed cell application the 1-Step Human Coupled In Vitro Translation kit (Pierce) was used. TelR15 coding sequences were subcloned from the mammalian expression plasmid into in vitro translation plasmid p7TCEF1-His and generated p7TCEF1-TelR15-mTagBP2, p7TCEF1-TelR15-mTFP1, p7TCEF1-TelR15-mGFPP, p7TCEF1-TelR15-YPet, and p7TCEF1-TelR15-mCherry. To produce TelR15 with green lysine incorporation, TelR15 or TelR15-mCherry were subcloned into the bacterial expression plasmid pET30a to generate pET30a-TelR15 and pET30a-TelR15-mCherry, and these plasmids were then used as DNA templates for coupled transcription–translation in the TnT T7 Quick Coupled kit (Promega) in the presence of green lysine (Promega). TALEColor plasmids are available at Addgene.

Telomere and Centromere Target Sequences of TALEColors. TALEs were designed to target the human telomere repeat (TTAGGG) regions on either strand. The forward telomere target sequence (TelF) was the 20-mer TAGGGTTAGGGTTAGGGTTA. The reverse telomere target sequences (TelR) were the 20-mer TAACCTAACCTAACCCTA, the 15-mer TAACCTAAACCCC-TAA, the 12-mer TAACCTAACCCT, the 9-mer TAACCTAA, and the 6-mer TAACCC. The pan-centromere target sequence, the chromosome 18-specific centromere target sequence, and the chromosome 15-specific centromere target sequence were TAGACAGAAGCATTCTCAGA, TTGAACCACCGTTTT-TAA, the 12-mer TAACCCTAACCC, the 9-mer TAACCTAA, and the 6-mer TAACCC.

Cell Culture and Transfection of TALEColors. The U2OS, HeLa 1.3 (11), HeLa S3, and IMR90 cells were cultured at 37 °C in Dulbecco-modified Eagle’s Minimal Essential Medium (DMEM; Life Technologies) supplemented with 10% (vol/vol) FBS. RPE1 cells (28) were cultured at 37 °C in DMEM:F12 medium supplemented with 10% (vol/vol) FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL) and then overlaid with mineral oil. A total of 50 ng of TALEColor plasmids were transfected using Lipofectamine 2000 (Life Technologies) and the cells were incubated for another 24 h. The microscope stage incubation chamber was maintained at 37 °C as described previously (29). Phase-contrast and fluorescence microscopy were performed with a Leica DM-IRB inverted microscope equipped with a mercury arc lamp, a 10-position filter wheel (Sutter Instrument), CFP/YFP/Red filter set, GFP/Red filter set (Semrock), a CCD camera (Photometrics), and MetaMorph acquisition software (Molecular Devices).

DNA Labeling by TALEColors in Fixed Cells. Cells grown on coverslips were fixed in ice-cold methanol for 10 min at −20 °C. All subsequent steps were carried out at room temperature. The fixed cells were incubated with 2N HCl for 5 min and then washed twice with PBS for 5 min each and then incubated with a given TALEColor probe as a 1:10 dilution from the coupled in vitro transcription–translation reaction mixtures for 30 min. The cells were then washed once with PBS for 5 min. Coverslips were mounted in Prolong Antifade (Molecular Probes), and images were captured with the fluorescence microscopy system described above.

Dual Labeling of Telomeres by TRF2 Immunofluorescence and TALEColors. Cells grown on coverslips were fixed in ice-cold methanol for 10 min at −20 °C. All subsequent steps were carried out at room temperature. Coverslips were incubated with TRF2 monoclonal antibodies (1:200 dilution; Millipore) in PBS-1% BSA for 1 h and followed by incubation together with TRITC-conjugated goat anti-mouse secondary antibody (Sigma) and in vitro translated TALEs: TelR15-mGFPP.

TALEColor Quantification and Single-Cell Imaging by Flow Cytometry. After labeling fixed cells with a given TALEColor probe as described above, they were trypsinized and centrifuged at 200 × g for 5 min and then washed (R1, light green, Top row, Far Right) and a moderate level (R2, dark green, Top row, Far Right), DRAQ5 positive cells with their low level of telomere labeling were sorted in parallel (R3, teal, Middle row, Far Right). (C) Representative DAPI images for HeLa 1.3 cells not labeled with TALEGreen-TelR15 (Far Left three columns), DAPI positive R1 cells (Center Left four columns), DAPI positive R2 cells (Center Right four columns), DRAQ5 positive R3 cells (Far Right four columns). BF, brightfield.
once with PBS. The cell concentration was adjusted to 1 x 10^7/mL in PBS and ice-cold methanol was then added to a final concentration of 90% (vol/vol) with gentle mixing. A total of 10^7 cells were resuspended in 100 μL of 2N HCl and incubated at 5 min at ambient temperature, then washed three times with 100 μL PBS (300 x g for 2 min). The cells were resuspended and DNA was labeled by adding 100 μL PBS containing 1 μg/mL of DAPI or DRAQ5 for 10 min and then washed twice with 100 μL PBS. Imaging flow cytometry was performed in the University of Massachusetts Medical School FACS Core Facility with an Amnis FlowSight imaging cytometer (Amnis). GFP was excited at 488 nm and its emission was collected in a 505- to 560-nm channel; DAPI was excited at 405 nm and its emission collected using a 430- to 505-nm filter. DRAQ5 was excited at 642 nm and its emission collected using a 642- to 740-nm filter. Flow cytometry and quantitative imaging data were acquired and analyzed by INSPIRE and IDEAS software (Amnis), respectively.

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