Submission ID #: 62173
Scriptwriter Name: Anastasia Gomez
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18962813

Title: Chemical Dimerization-Induced Protein Condensates on Telomeres

Authors and Affiliations:

Rongwei Zhao[1], David M. Chenoweth[2], Huaiying Zhang[1]*

[1]Department of Biological Sciences, Mellon College of Science, Carnegie Mellon University, Pittsburgh, PA, United States
[2]Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, United States

Corresponding Authors:

Huaiying Zhang (huaiyinz@andrew.cmu.edu)

Email Addresses for All Authors:

rongweiz@andrew.cmu.edu
dcheno@sas.upenn.edu
huaiyinz@andrew.cmu.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **NO.**

Current Protocol Length

Number of Steps: 23
Number of Shots: 42

Introduction

1. Introductory Interview Statements

1.1. This protocol describes a chemical dimerization system to induce protein condensates on telomers. It can be easily adapted to induce condensates on other genomic loci and is suitable for probing the formation and function of chromatin-associated condensates.

1.1.1. LAB MEDIA: Figure 1 A.

1.2. This method offers temporal resolution required for live cell imaging and maintains phase separation in a population of cells for biochemical assays.

1.2.1. *5.1.1.*

Protocol

2. Dimerization on telomeres

2.1. To begin, dissolve dimerizers in DMSO at 10 millimolar **[1]** and store them in plastic microcentrifuge tubes at −80 degrees Celsius for long-term storage **[2]**.

2.1.1. Talent dissolving dimerizers in DMSO.

2.1.2. Talent placing the tubes in the freezer.

2.2. Dilute an aliquot of 10 millimolar dimerizer in imaging medium to a stock concentration of 10 micromolar **[1]** and store it at −20 degrees Celsius **[2]**.

2.2.1. Talent diluting dimerizer in imaging medium.

2.2.2. Talent placing the diluted dimerizer in a freezer.

2.3. When ready to use, dilute dimerizers to a final working concentration of 100 nanomolar in growth medium or imaging medium **[1]**.

2.3.1. Talent dissolving the dimerizer in growth medium.

3. Immunofluorescence (IF)

3.1. Seed 10^5 cells on 12-millimeter diameter circular cover glasses coated with poly-D-lysine in a 6-well plate **[1]**, then transfect the cells with the Halo-GFP-TRF1 *(pronounce 'halo-G-FP-T-R-F-one')* and mCherry-eDHFR-SIM *(pronounce 'm-cherry-e-D-H-F-R-sim')* or mCherry-eDHFR-SIM mutant plasmids and wait for 24 to 48 hours before proceeding to immunofluorescence **[2]**.

3.1.1. Talent seeding cells on a coverslip.

3.1.2. Talent adding plasmids to the cells.

3.2. Add the diluted 100 nanomolar dimerizers to the cells **[1]** and incubate them at 37 degrees Celsius for 4 to 5 hours **[2]**. *Videographer: This step is important!*

3.2.1. Talent adding dimerizers to the cells.

Added shot: Talent wrapping aluminum foil outside the plate.

3.2.2. Talent placing the coverslips in the incubator.

3.3. After the incubation, fix the cells in PBS solution containing 4% formaldehyde and 0.1% Triton X-100 for 10 minutes at room temperature to permeabilize the cells **[1]**, then wash cells three times with PBS **[2]**.

3.3.1. Talent adding fixative to the cells.

Added shot: Talent removing fixative and transferring the coverslips on a parafilm.

3.3.2. Talent washing the coverslips in PBS.

3.4. Wash coverslips twice with 50 microliters of TBS-Tx and once with 50 microliters of Antibody Dilution Buffer **[1-TXT]**. Incubate each coverslip with 50 microliters of primary anti-PML, anti-SUMO1, or anti-SUMO2/3 *(pronounce 'SUMO-two and three')* antibody at 4 degrees Celsius in a humidified chamber overnight **[2]**.

3.4.1. Talent washing coverslips with TBS-Tx or Antibody Dilution Buffer. **TEXT: TBS-Tx: TBS, 0.1% Triton X-100, 0.05% Na-azide; Antibody Dilution Buffer: TBS-Tx, 2% BSA, 0.05% Na-azide**

3.4.2. Talent placing the coverslips with the antibodies in the humidified chamber.

3.5. Wash coverslips three times with Antibody Dilution Buffer to remove unbound primary antibody **[1]**, then incubate the cells with secondary antibody for 1 hour in a dark box at room temperature **[2-TXT]**.

3.5.1. Talent washing coverslips with Antibody Dilution Buffer.

3.5.2. Talent adding secondary antibody to coverslips. **TEXT: See Text Manuscript for secondary antibodies**

3.6. After staining, wash coverslips three times with TBS-Tx **[1]**. Label slides by adding 2 microliters of 1 microgram per milliliter DAPI **[2]**, then flip the coverslips over and place them onto the DAPI drop **[3]**. Aspirate extra fluid from the edge of the coverslip **[4]**.

3.6.1. Talent washing the coverslips with TBS-Tx.

3.6.2. Talent adding DAPI to slides.

3.6.3. Talent flipping the coverslips onto the slides.

3.6.4. Talent aspirating excess fluid.

3.7. Seal the slide with nail polish, allow it to dry **[1]**, and rinse the top of the coverslip with water **[2]**. Place the slides in the freezer until imaging **[3]**.

3.7.1. Talent sealing a slide with nail polish.

3.7.2. Talent rinsing the top of the coverslip.

3.7.3. Talent placing the slides in the freezer.

4. Fluorescence in situ hybridization (FISH)

4.1. Seed 10^5 cells on 12-millimeter circular cover glasses coated with poly-D-lysine in a 6-well plate and transfected them with Halo-TRF1 and mCherry-eDHFR-SIM or mCherry-eDHFR-SIM mutant plasmids **[1]**.

4.1.1. Talent seeding cells onto a coverslip.

4.2. Fix the cells with 4% formaldehyde for 10 minutes at room temperature **[1]** and wash them four times with PBS **[2]**.

4.2.1. Talent adding 4% formaldehyde to the cells.

4.2.2. Talent washing the coverslips.

4.3. For IF-FISH *(pronounce 'I-F-fish')* , stain the cells with primary and secondary antibodies and refix them with 4% formaldehyde for 10 minutes at room temperature **[1]**, then wash them four times with PBS **[2]** and dehydrate the coverslips in an ethanol series **[3-TXT]**.

4.3.1. Talent refixing the cells with formaldehyde.

4.3.2. Talent washing the coverslips with PBS.

4.3.3. Talent washing the coverslips with ethanol. **TEXT: 70%, 80%, 90%; 2 min each**

4.4. Incubate the coverslips with the 488-telC PNA *(pronounce 'four-eighty-eight-telomere-C-P-N-A')* probe in 5 microliters of hybridization solution at 75 degrees Celsius for 5 minutes **[1]**. Then, incubate the coverslips overnight in a humidified chamber at room temperature **[2]**.

Added shot: Talent adding the PNA probe on the slide and flipping the coverslips onto the PNA probe droplets.

4.4.1. Talent placing the coverslips with the PNA probe into an incubator.

4.4.2. Talent putting the coverslips in the humidified chamber.

4.5. Wash the coverslips three times with wash buffer for 2 minutes per wash at room temperature **[1]** and mount them with 1 microgram per milliliter DAPI in mounting media for imaging **[2]**.

4.5.1. Talent washing the coverslips with wash buffer.

4.5.2. Talent mounting the coverslips.

5. Imaging

5.1. For live imaging, mount the coverslips in magnetic chambers on a heated stage in an environmental chamber, maintaining the cells in 1 milliliter of imaging medium without phenol red **[1]**.

5.1.1. Talent mounting the coverslips onto the stage.

5.2. Set up the microscope and environmental control apparatus as described in the text manuscript **[1]**. *Videographer: This step is important!*

5.2.1. Microscope and environmental control apparatus.

5.3. Locate cells with a bright GFP signal on the telomeres and diffusive mCherry signal in the cytosol. Find around 20 cells, memorizing each position with the x,y, z information, and set up parameters for time lapse imaging **[1]**.

5.3.1. Talent locating the cells and memorizing their position.

5.4. Use 0.5-micrometer spacing for a total of 8 micrometers in Z and a 5-minute time interval for 2 to 4 hours for both GFP and mCherry channels. Use 30% of 594-nanometer and 50% of 488-nanometer power intensity, with exposure times of 200 milliseconds and a camera gain of 300 **[1]**.

5.4.1. SCREEN: 5-4-1_Screenshot1.mp4. 00:56 – 01:09, 00:24 – 00:25, 00:28 – 00:43, 00:04 – 00:13 *Videographer: Film the screen as a backup for all SCREEN shots*

Video editor: Speed up the video between 00:28 – 00:43.

Video editor: Speed up the video between 00:56 – 01:09.

5.5. Start imaging and take one-time loop as pre-dimerization **[1]**. Then, pause imaging, add 0.5 milliliters of imaging media containing 15 microliters of 10 micromolar dimerizer to the imaging chamber, taking care to not touch the stage **[2]**, and resume imaging **[3]**. *Videographer: This step is difficult and important!*

5.5.1. SCREEN: 5-5-1_Screenshot2.mp4. 00:00 – 00:02, 00:19 – 00:20, 0:26 – 00:27, 02:31 – 02:33.

5.5.2. Talent adding imaging media with dimerizer to the imaging chamber.

5.5.3. Talent resuming imaging.

5.6. When ready to reverse dimerization, pause imaging **[1]** and add 0.5 milliliters of imaging media containing 2 microliters of 100 millimolar stock TMP to the imaging chamber **[2]**. Continue imaging the cells for 1 to 2 hours **[3]**.

5.6.1. Talent pausing imaging.

5.6.2. Talent adding imaging media with TMP to the imaging chamber.

5.6.3. Talent resuming imaging.

5.7. For fixed imaging, use the same microscope set up as live imaging, but without the stage heating. Locate around 30 to 50 cells with the red signal to select for transfected cells **[1]**.

5.7.1. Talent locating the cells with the red signal.

5.8. Acquire images with 0.3-micrometer spacing for a total of 8 micrometers in Z. Use 80% of 647-nanometer, 80% of 561-nanometer, and 70% of 488-nanometer power intensity, with exposure times of 600 milliseconds and a camera gain of 300 **[1]**.

5.8.1. SCREEN: 5-8-1_Screenshot3.mp4. 00:03 – 00:04, 00:08 – 00:22, 00:33 – 00:38, 00:54 – 00:59. *Video editor: Speed up the video between 00:08 – 00:22*

Results

6. Results: Chemical dimerization to induce chromatin-associated condensates

6.1. Telomeric localization of SUMO was identified using telomere DNA FISH and SUMO protein immunofluorescence **[1]**.

6.1.1. LAB MEDIA: Figure 2 B – E.

6.2. Cells with SIM *(pronounce 'sim')* recruitment enriched SUMO1 and SUMO 2/3 *(pronounce 'SUMO-two and three')* **[1]** compared to cells with SIM mutant recruitment **[2]**, indicating that SIM dimerization-induced SUMO enrichment on telomeres depends on SUMO-SIM interactions **[3]**.

6.2.1. LAB MEDIA: Figure 2 B – E. *Video Editor: Emphasize B and D.*

6.2.2. LAB MEDIA: Figure 2 B – E. *Video Editor: Emphasize C and E.*

6.2.3. LAB MEDIA: Figure 2 B – E.

6.3. A time lapse movie of TRF1 and SIM after dimerization is shown here. SIM was successfully recruited to telomeres and both SIM and TRF1 foci became larger and brighter, as predicted for liquid droplet formation and growth **[1]**.

6.3.1. LAB MEDIA: 6-3-1_Lab Media.mp4. *Video Editor: Speed this up as necessary.*

6.4. In addition, fusion of TRF1 foci was observed **[1]**, which led to telomere clustering as shown in the reduced telomere number **[2]** and increased telomere intensity over time **[3]**.

6.4.1. LAB MEDIA: Figure 3 B.

6.4.2. LAB MEDIA: Figure 3 E.

6.4.3. LAB MEDIA: Figure 3 D.

6.5. In contrast, SIM mutant was recruited to telomeres after dimerization but did not induce any droplet formation or telomere clustering, indicating that phase separation and telomere clustering is driven by SUMO-SIM interactions **[1]**.

6.5.1. LAB MEDIA: 6-5-1_Lab Media.mp4. *Video Editor: Speed this up as necessary.*

6.6. The reversal of phase separation and telomere clustering was observed after adding excess free TMP **[1]**. Telomere number increased and telomere intensity decreased over time **[2]**.

6.6.1. LAB MEDIA: 6-6-1_Lab Media.mp4. *Video Editor: Speed this up as necessary.*

6.6.2. LAB MEDIA: Figure 4 B and C.

6.7. Representative images of APBs identified by telomere DNA FISH and PML protein IF are shown here **[1]**. Cells with SIM recruited have more APBs **[2]** than cells with SIM mutant recruited, suggesting dimerization-induced condensates are indeed APBs **[3]**.

6.7.1. LAB MEDIA: Figure 5.

6.7.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize A.*

6.7.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize B.*

Conclusion

7. Conclusion Interview Statements

7.1. When performing this protocol, do not expose light sensitive dimerizers to light. Work in a dark room with a lamp that emits red light and wrap cells with aluminum foil during incubation.

7.1.1. *5.2.*

7.2. Following this procedure, proximity labeling can be used to generate a comprehensive list of components in the induced condensate. Live imaging can be used to follow the dynamics of components in the condensate. These methods can help determine the roles of governing condensate composition control.

7.2.1. *5.7.1.*