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Title: Defining the Program of Maternal mRNA Translation During In Vitro Maturation Using a Single Oocyte Reporter Assay

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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? **YES**

   If **Yes**, can you record movies/images using your own microscope camera?

   NO

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Olympus SZ60

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

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**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

   This will be done for the author statement of Ria Marathe

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option.

   This will be done for the author statements of Natasja Costermans and Enrico Daldello

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

Current Protocol Length

Number of Steps: 18
Number of Shots: 45

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Natasja Costermans:** This method may be used to identify and characterize regulatory elements that control translation during oocyte meiotic maturation and that are critical for oocyte developmental competence.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Ria Marathe:** We apply time-lapse microscopy to assess reporter accumulation throughout *in vitro* oocyte maturation. This accurately pinpoints the time when translational activation or repression takes place during oocyte maturation.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

1.3. The experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of California at San Francisco.

Protocol

2. Aspiration of denuded oocyte and its microinjection

2.1. To begin, carefully open the antral follicles by making a small cut in the follicle wall with a 26-gauge needle **[1]**. Isolate intact COCs with several layers of cumulus cells using a mouth-operated glass pipette **[2-TXT]**. Use a smaller pipette and mechanically denude the COCs by repeated pipetting **[3-TXT]**.

2.1.1. SCOPE: 2.1.1_opening_the_antral_follicle.MOV. 0:25 – 0:35.

2.1.2. SCOPE: 2.1.2_isolating_COCs.MOV. 0:05 – 0:10. **TEXT: COCs- cumulus-enclosed oocytes.**

2.1.3. SCOPE: 2.1.3_mechanical_denuding_COCs.MOV. 0:20 – 0:25. **TEXT: Alternatively, perform micro-injection on intact COCs.**

2.2. Aspirate the denuded oocytes **[1]** and place them in a Petri dish with maturation medium supplemented with 1 micromolar of cilostamide **[2-TXT]**. Place the dish in an incubator for 2 hours to let the oocytes recover from the stress induced by their isolation from the follicles **[3]**.

2.2.1. Talent aspirating the denuded oocytes using large pipette.

2.2.2. Talent placing the oocyte in petri dish with cilostamide. **TEXT: Cilostamide is a phosphodiesterase inhibitor which prevents resumption of meiosis.**

2.2.3. Talent placing the dish in the incubator.

2.3. Place a 10-centimeter-long borosilicate glass capillary tube in a mechanical puller to prepare injection needles **[1]**. Bend the needle tip at a 45-degree angle using a heated filament for optimal injection **[2]**.

2.3.1. LAB MEDIA: Step 2.3.1 Mechanical puller.MOV. *Video editor play from 00:01 to 00:19*

2.3.2. LAB MEDIA: Step 2.3.2 Bending the needle part 2.MOV. *Video editor play the complete video.*

2.4. Place 20-microliter droplets of basic oocyte collection medium in a polystyrene dish **[1]** and cover the droplets with light mineral oil **[2]**.

2.4.1. Talent placing drops of oocyte collection medium in polystyrene dishes.

2.4.2. Talent covering the droplets with mineral oil.

2.5. Prepare a larger volume of reporter mix by adding 12.5 micrograms per microliter of Ypet-3' *(why-pet)* UTR and mCherry each **[1-TXT]**. Store aliquots of these at -80 degrees Celsius **[2]**. Upon thawing, centrifuge the aliquot for 2 minutes at 20,000 times *g* **[3]**, then transfer it to a new microcentrifuge tube **[4]**.

2.5.1. Talent adding Ypet-3' UTR and mCherry. **TEXT: UTR-untranslated region.**

2.5.2. Talent storing the aliquots in -80 degrees.

2.5.3. Talent centrifuging the aliquot.

2.5.4. Talent transferring the supernatant to a new tube.

2.6. Load the injection needle with approximately 0.5 microliters of reporter mix **[1]**. Place the holding pipette and the injection needle into the holders **[2]** and position them in the droplet of oocyte collection medium **[3]**. *Videographer: This step is important!*

2.6.1. Talent loading the injection needle with reporter mix.

2.6.2. Talent placing the holding pipette and injection needle into the holders.

2.6.3. Talent positioning the needle in the droplet of medium.

2.7. Open the injection needle by gently tapping it against the holding pipette **[1]**. Place oocytes in a droplet of basic collection medium **[2]** and inject 5 to 10 picoliters of the reporter mix **[3]**. Incubate the oocytes in the maturation medium with 1 micromolar cilostamide for 16 hours to allow the mCherry signal to plateau **[4]**. *Videographer: This step is important!*

2.7.1. LAB MEDIA: 2.7.1 Opening injection needle.MOV. *Video editor play the video from 00:01 to 00:11.*

2.7.2. Talent placing the oocyte in a droplet of collection medium.

2.7.3. LAB MEDIA: 2.7.3 Oocyte injection.MOV. *Video editor play the video from 00:01 to 00:07.*

2.7.4. Talent incubating the oocyte in maturation medium.

2.8. For time-lapse microscopy, prepare a Petri dish with two 20-microliter droplets of maturation medium for each injected reporter; one droplet with 1 micromolar cilostamide for control prophase I *('one')*-arrested oocytes and one droplet without cilostamide for maturing oocytes **[1]**.

2.8.1. Talent placing two 20 microliters droplets of maturation medium into the Petri dish.

2.9. Cover the droplets with light mineral oil **[1]** and place them in the incubator **[2]**.

2.9.1. Talent covering the droplets with mineral oil.

2.9.2. Talent placing the dishes in incubator.

3. Time-lapse microscopy and Analysis of Ypet-3' UTR translation

3.1. After pre-incubation, remove the injected oocytes from the incubator **[1]**, and wash them four times in maturation medium without cilostamide **[2]**. Keep some oocytes in maturation medium with 1 micromolar cilostamide as a prophase I *('one')* oocyte control group **[3]**.

3.1.1. Talent removing the oocytes from the incubator.

3.1.2. Talent washing the oocytes in maturation medium.

3.1.3. Talent keeping some oocytes in maturation medium with cilostamide as control group.

3.2. Transfer the injected oocytes to their respective droplets on the previously prepared time-lapse microscope dish **[1]**. Cluster the oocytes with a closed glass pipette to prevent their movement during the recording **[2]**.

3.2.1. Talent transferring the oocytes to their respective droplets.

3.2.2. Talent clustering the oocytes. **TEXT: Prepare a closed pipette by holding the tip in a flame for a few seconds**

3.3. Place the dish under the microscope equipped with a light-emitting diode illumination system and a motorized stage equipped with an environmental chamber maintained at 37 degrees Celsius and 5% carbon dioxide, using the parameters mentioned in the text manuscript **[1]**.

3.3.1. Talent placing the dish under microscope.

3.4. Enter the appropriate settings for the time-lapse experiment by clicking on **Apps** and then **Multi Dimensional Acquisition [1]**. Select the first tab **Main** and select **timelapse**, **multiple stage positions**, and **multiple wavelengths [2]**.

3.4.1. SCREEN: SCREEN62041_3.6 Multi Dimensional Acquisition.mp4. 00:07 to 00:10.

3.4.2. SCREEN: SCREEN62041_3.6 Multi Dimensional Acquisition.mp4. 00:10 to 00:12.

3.5. Select the tab **Saving** to enter the location where the experiment should be saved **[1]**, then select the tab **Timelapse** to enter the number of time points, duration, and time interval **[2]**.

3.5.1. SCREEN: SCREEN 62041_3.7 Saving Timelapse.mp4. 00:01 to 00:16.

3.5.2. SCREEN: SCREEN 62041_3.7 Saving Timelapse.mp4. 00:17 to 00:33.

3.6. Select the tab **Stage [1]**. Switch on brightfield **[2]** and locate the position of the oocytes by opening a new window and selecting **Acquire**, **Acquire**, and **Show Live [3]**. Once the oocytes are located, switch back to the **Multi Dimensional Acquisition** window, and press **plus** to set the location of the oocytes **[4]**.

3.6.1. SCREEN: SCREEN 62041_3.8 Setting Stage and Locating Oocyte.mp4. 00:01 to 00:08.

3.6.2. SCREEN: SCREEN 62041_3.8 Setting Stage and Locating Oocyte.mp4. 00:09 to 00:18.

3.6.3. SCREEN: SCREEN 62041_3.8 Setting Stage and Locating Oocyte.mp4. 00:18 to 00:31.

3.6.4. SCREEN: SCREEN 62041_3.8 Setting Stage and Locating Oocyte.mp4. 00:31 to 00:32. *Video editor play the video show clicking on +*

3.7. Select the tab **Wavelengths** and set 3 different wavelengths for brightfield, YFP, and mCherry. Adjust the exposure for Ypet and mCherry **[1]**, then start the time lapse experiment by clicking on **Acquire [2]**.

3.7.1. SCREEN: SCREEN 62041_3.9 Setting Wavelength and Acquire.mp4. 00:01 to 00:46.

3.7.2. SCREEN: SCREEN 62041_3.9 Setting Wavelength and Acquire.mp4. 00:47 to 00:59.

3.8. For analysis of Ypet-3' UTR translation, perform two region measurements for each oocyte; the oocyte itself by clicking on **ellipse region [1-TXT]** and a small region surrounding the oocyte to be used for background subtraction by click on **rectangular region [2]**.

3.8.1. SCREEN: SCREEN 62041_3.10 and 3.11 Performing Oocyte Measurement and Exporting Data (1).mp4 00:07 to 00:13. **TEXT: oocytes must remain in the selected region during recording**

3.8.2. SCREEN: SCREEN 62041_3.10 and 3.11 Performing Oocyte Measurement and Exporting Data (1).mp4 00:14 to 00:17.

3.9. Export the region measurement data to a spreadsheet by clicking **Open Log** and then **Log Data [1]**. For each individual oocyte and for all measured time-points, subtract the background region measurement from the oocyte region measurement, separately for YFP and mCherry wavelengths **[2]**.

3.9.1. SCREEN: SCREEN 62041_3.10 and 3.11 Performing Oocyte Measurement and Exporting Data (1).mp4 00:31 to 00:55.

Results

4. Statistical analysis of the expression for reporter-YFP and mCherry in prophase I and maturing oocytes

4.1. The expression of mCherry and YFP in prophase I *('one')* and maturing oocytes was recorded in 39 oocytes, of which 30 were matured, and 9 were arrested in prophase I as a negative control **[1]**.

4.1.1. LAB MEDIA: Figure 3.

4.2. Individual YFP to mCherry ratios for signals of prophase I-arrested **[1]** and maturing oocytes **[2]** were corrected for injected volume by dividing the YFP signal by the average mCherry signal of the last 10 time-points for prophase I-arrested **[3]** and maturing oocytes **[4]**.

4.2.1. LAB MEDIA: Figure 4A. *Video editor focus on the stationary graph.*

4.2.2. LAB MEDIA: Figure 4B. *Video editor focus on the gradual increase in the graph after cilostamide release.*

4.2.3. LAB MEDIA: Figure 4C. *Video editor focus on the stationary line graph.*

4.2.4. LAB MEDIA: Figure 4D. *Video editor focus on the gradual increase in the mean line graph after cilostamide release.*

4.3. Translation rates of the reporter-YFP were measured by curve-fitting the YFP to mCherry ratios in prophase I and in maturing oocytes during the first 0 to 2 hours or 8 to 10 hours after cilostamide release using linear regression **[1]**.

4.3.1. LAB MEDIA: Figure 5A.

4.4. The accumulation of the reporter does not follow a linear pattern, as indicated by a significant difference in translation rates between 0 to 2 hours and 8 to 10 hours after cilostamide release, indicating activation of Interleukin-7 translation during oocyte meiotic maturation **[1]**.

4.4.1. LAB MEDIA: Figure 5B. *Video editor first focus on the black dots on the 0-2 bar graph and then on 8-10 bar graph.*

Conclusion

5. Conclusion Interview Statements

5.1. **Enrico Daldello:** When attempting this protocol, make sure that the exposure time is adequate at the beginning of the recording. Keep in mind that the signal might saturate during the time-course if translation is activated during oocyte meiotic maturation.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7.2.*

5.2. **To be determined:** Stability of the mRNA encoding the reporter can be measured by qPCR using primers specific for YFP. Western blotting using an antibody against the V5 tag can be used to confirm changes in translation of the reporter.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. NOTE: Authors didn't indicate who said it. If this was recorded, please use the shot to determine who it was. If you can't, please let me know and I will check with the authors.