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Title: Imaging Intranuclear Actin Rods in Live Heat Stressed Drosophila Embryos

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes.**

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

   No. We cannot record movies/images on our dissection or microinjection scopes.

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope. **The dissection microscope is a Zeiss Stemi 508. The microinjection scope is a Zeiss Invertoskop 40 C.**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No.**

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

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Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Natalie Biel:** Our protocol is significant because it uses the well-established technique of microinjection in *Drosophila* embryos to allow new research on the Actin Stress Response and accompanying actin rod assembly.

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

1.2. **Natalie Biel:** The main advantage of this technique is that we can study the Actin Stress Response in a variety of contexts, such as a mutant screen or under different stress conditions.

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

OPTIONAL:

1.3. **Natalie Biel:** An individual who has never performed this technique before may struggle with handling the embryos and performing the microinjection. We recommend practicing these steps until the user feels comfortable.

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

1.4. **Natalie Biel:** Visual demonstration of this method is critical because the steps of embryo handling, positioning, and mounting for both microinjection and imaging might not be clear from written instructions alone.

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

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Protocol

2. Embryo Collection and Mounting

2.1. Prepare embryo collection cups and apple juice agar plates according to manuscript directions **[1]**. To promote the most generous egg laying, set up collection cups with flies 2 days prior to the experiment. Add at least 100 females and 50 male flies to each collection cup **[2]**, then cover it with an apple juice plate **[3]**.

2.1.1. WIDE: Establishing shot of talent walking to the lab bench with collection cups and apple juice agar plates in hand.

2.1.2. Talent adding flies to the collection cup.

2.1.3. Talent covering a collection cup with a plate.

2.2. Prepare a working solution of G-actin-red and store it on ice until it is loaded into the microneedle **[1-TXT]**. Allow flies to lay embryos for 30 minutes on the apple juice plates with yeast paste at 18 degrees Celsius **[2]**.

2.2.1. Talent placing the labeled container of G-actin on ice. **TEXT: Store on ice for up to 6 hours**

2.2.2. Flies in the collection cups.

2.3. While the flies are laying, cut out a 4 by 1-centimeter rectangular wedge of apple juice agar with a razor blade **[1]**, and place it on a 25 by 75-millimeter glass slide **[2]**. Harvest the plate from the collection and dechorionate the embryos by pouring fresh bleach solution onto the plate and swirling it for 1 minute **[3-TXT]**.

2.3.1. Talent cutting the agar.

2.3.2. Talent placing the agar on the slide.

2.3.3. Talent pouring the bleach solution on the plate and swirling it. **TEXT: 1:1 bleach : distilled water**

2.4. Dampen the bristles of a paint brush with distilled water **[1]**, then use it to transfer dechorionated and washed embryos from the collection basket onto the prepared apple juice agar wedge on the glass slide **[2]**.

2.4.1. Talent wetting the brush.

2.4.2. SCOPE: Talent transferring the embryos from the collection basket to the ~~slide~~ agar.

2.5. Use a pair of fine-tip tweezers or a dissecting needle to arrange ten embryos in a straight line along the long axis of the rectangular agar wedge. Arrange the embryos head-to-tail, such that their dorsal side is facing the researcher **[1]**. *Videographer: This step is important!*

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2.5.1. SCOPE: Talent arranging the embryos.

2.6. Cut half of a centimeter off of the end of a P200 pipette tip **[1]** with a razor blade and dip it into the embryo glue **[2]**. Generously coat a 5-millimeter region along the long edge of a 24 by 50-millimeter rectangular coverslip and let it dry, glue side up **[3]**.

2.6.1. Talent cutting the pipette tip.

2.6.2. Talent dipping the tip into the embryo glue.

2.6.3. Talent coating the edge of the coverslip with the glue.

2.7. Once the embryo glue has dried, gently place the coverslip glue side down on top of the row of aligned embryos on the agar, leaving 2 to 3 millimeters of space between the edge of the coverslip and the row of embryos **[1]**.

2.7.1. Talent placing the coverslip on the row of aligned embryos on the agar. NOTE: 2.7.1 and 2.8.1 together

2.8. Flip the coverslip over so that the embryos are facing up **[1]**. They should be stuck in a line along one long edge of the coverslip, with their ventral region facing the closest edge **[2]**.

2.8.1. Talent flipping the coverslip.

2.8.2. SCOPE: Flipped coverslip with the embryos aligned along the edge.

2.9. Place the coverslip on top of 150 grams of fresh blue desiccant in a 16-ounce screw top jar **[1]** and tightly screw on the lid. Desiccate the embryos for 8 to 10 minutes **[2]**. Then, remove the coverslip from the desiccant jar **[3]** and tape each short side of the coverslip to a microscope slide, embryo side up **[4]**.

2.9.1. Talent putting the coverslip in the jar.

2.9.2. Talent screwing on the lid and leaving the jar on the lab bench.

2.9.3. Talent taking the coverslip out of the jar.

2.9.4. Talent taping the coverslip to the slide.

2.10. Add 2 to 3 drops of Halocarbon 27 oil with a Pasteur pipette to cover the aligned embryos and protect them from further dehydration **[1]**.

2.10.1. Talent adding oil to the embryos.

3. Injection and Heat Stress to Promote Rod Formation

3.1. While embryos are desiccating, backload the previously prepared G-actin-red supernatant into the microneedle with a micro loader tip **[1]**. Attach the microneedle to the needle holder and tighten the screw **[2]**.

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3.1.1. Talent backloading the microneedle with the G-actin^{red}. NOTE: Added an additional shot for this step; choose WIDE or CLOSE-UP, or show both for this step.

3.1.2. Talent attaching the microneedle to the needle holder and tightening the screw.

3.2. Place the slide with mounted embryos onto the microscope stage **[1]** and use the microneedle controls to bring the needle into the same focal plane as the embryos **[2]**.

3.2.1. Talent placing the slide on the microscope stage.

3.2.2. SCOPE: Needle coming into focus.

3.3. Insert the microneedle into the embryo so that it hits the embryo in the middle of its ventral region, at the embryo equator **[1]**. When the microneedle tip is visible inside the middle of the embryo, trigger the injection **[2]**. *Videographer: This step is difficult and important!*

3.3.1. SCOPE: Microneedle inserting into the embryo.

3.3.2. SCOPE: Embryo being injected.

3.4. After the injection, slowly remove the microneedle **[1]**. Move the stage and perform the injection on the other embryos **[2]**. After all embryos have been injected, place the slide with the embryos in a humid incubation chamber and close the lid **[3]**.

3.4.1. SCOPE: Microneedle being removed.

3.4.2. SCOPE: Stage moving.

3.4.3. Talent placing the slide in the humid incubation chamber.

4. Imaging of Actin Rods with Confocal Microscopy

4.1. Remove the slide with the injected embryos from the humid incubation chamber **[1]**. Working quickly, gently pry off the double-sided tape pieces that were used to adhere the coverslip to the slide **[2]**.

4.1.1. Talent taking a slide from the humid incubation chamber.

4.1.2. Talent taking the tape off of the coverslip and slide.

4.2. Stick two 2.5-centimeter long pieces of double-sided tape together and cut the tape in half lengthwise to make two strips **[1]**. Stick two-thirds of the length of each tape strip onto the first coverslip, flanking each side of the embryos. Leave one-third of the tape strips hanging off the edge of the first coverslip where the embryos are stuck **[2]**. *Videographer: This step is important!*

4.2.1. Talent sticking the pieces of tape together and cutting them.

4.2.2. Talent sticking 2/3 of the length of each tape strip onto the coverslip.

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4.3. Gently place a second rectangular coverslip on top of the tape strips to sandwich the embryos between the coverslips, aligning the 25-millimeter edges but keeping the 50-millimeter edges offset from one another by 1 centimeter **[1]**. *Videographer: This step is important!*

4.3.1. Talent sandwiching the embryos between the two coverslips, with the coverslip edges properly aligned.

4.4. Confirm that the heated stage is at the appropriate temperature **[1]** and, if using an inverted microscope, add immersion liquid onto the selected objective lens **[2]**. Place the coverslip sandwich onto the stage carefully, making sure that the new imaging surface is the one touching the immersion liquid **[3]**.

4.4.1. Talent confirming the temperature of the stage.

4.4.2. Talent adding immersion liquid onto the lens.

4.4.3. Talent positioning the coverslip sandwich on the microscope stage.

4.5. Focus on an embryo that is in cellularization. Then, switch to the laser acquisition mode on the confocal microscope and adjust laser power and gain, frame size, tiling, and projection settings as desired **[1]**.

4.5.1. LAB MEDIA: 4-5-1_EmbryoInCellularization.ai. Still image of an embryo in cellularization, to be submitted by authors.

4.6. Take surface-view images through the focal planes of the embryo's nuclei to find intranuclear actin rods **[1]**. Rods should appear in multiple orientations as bright streaks or dots inside the comparatively dark nuclei **[2]**.

4.6.1. LAB MEDIA: 4-6-1_ActinRods_In_FocalPlanes.ai. Still image showing intranuclear actin rods in different focal planes, to be submitted by authors.

4.6.2. LAB MEDIA: Figure 2 A, image on the right only.

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Results

5. Results: Actin Rods in Heat-stressed Embryos

5.1. The actin stress response in heat shocked embryos is evident by the assembly of intranuclear actin rods **[1]**. Actin rods appear in different orientations inside the nuclei and can be imaged through several focal planes **[2]**.

5.1.1. LAB MEDIA: Figure 2 A.

5.1.2. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the image on the right.*

5.2. In comparison, control embryos incubated at 18 degrees Celsius do not display actin rods **[1]**. The percent nuclei containing rods can be quantified **[2]** and FRAP experiments can be performed on the rods **[3]**.

5.2.1. LAB MEDIA: Figure 2 A. *Video Editor: Emphasize the image on the left.*

5.2.2. LAB MEDIA: Figure 2 B.

5.2.3. LAB MEDIA: Figure 2 C.

5.3. An example of a fluorescence recovery plot for a bleached versus unbleached region of an actin rod is shown here **[1]**.

5.3.1. LAB MEDIA: Figure 2 D.

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Conclusion

6. Conclusion Interview Statements

6.1. **Natalie Biel:** When attempting this procedure, it is important to remember that actin rod formation is temperature dependent. For the steps following microinjection, it is crucial to keep embryos at the desired incubation temperature.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.3 or 4.4.1.*

6.2. **Natalie Biel:** After microinjection, the researcher can perform FRAP to confirm the lack of F-actin turnover within actin rods, or to quantify changes in F-actin turnover occurring in other actin-rich structures.

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

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