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Title: Quantifying Tissue-Specific Proteostatic Decline in *Caenorhabditis elegans*

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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?

Yes

- Leica MZFLIII with a Pixelink video-capable camera (we already have example video files as recorded through this microscope, and we can record additional video if needed)
- Zeiss Imager.M2m (we intend to record video of using the microscope, but not video through this microscope)

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**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Maria Lazaro-Pena:** The use of tissue-specific expression of a polyglutamine fluorescent reporter in *C. elegans* is significant because it allows the discovery and characterization of proteostasis regulators in the context of an intact multicellular organism.

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

1.2. **Adam Cornwell:** The main advantage of this technique is that it allows visualization and quantification of the age-associated decline in cellular proteostasis *in vivo*, which is essential to gain deeper mechanistic insight into how organisms maintain proper folding and function of the proteome, and the effects of aging.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.2.1.*

OPTIONAL:

1.3. **Andrew Samuelson:** Elucidating mechanisms capable of preserving proteostasis will facilitate the development of targeted interventions for the treatment of aging-associated diseases in which proteostasis is compromised, and to promote healthy aging.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.8.1.*

1.4. **Maria Lazaro-Pena:** Proteostasis collapse is a massive clinical problem as it underlies the development of protein misfolding diseases, including Alzheimer's, Parkinson's, Huntington's Disease and Amyotrophic lateral sclerosis.

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

Introduction of Demonstrator on Camera

1.5. **Maria Lazaro-Pena:** Demonstrating the procedure will be Sifan, a graduate student from our laboratory.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Reagent Preparation

2.1. Begin by using 6-centimeter diameter Petri plates to prepare 5 agar plates for each test condition **[1]**. For experiments with RNAi, induce dsRNA production in transformed HT115 *E. coli* and use RNAi agar **[2]**. Use OP50 *E. coli* on standard NGM agar for experiments without RNAi **[3]**.

2.1.1. WIDE: Establishing shot of talent preparing plates at the lab bench.

2.1.2. Stack of plates.

2.1.3. Added shot: Talent inoculating *E. coli* into LB Medium

2.2. Grow the E. coli cultures overnight at 37 degrees Celsius while shaking at 220 rpm **[1-TXT]**. On the next day, pellet the bacteria by centrifugation at 2,400 *g* for 15 to 20 minutes **[2]**, then aspirate the supernatant **[3]** and resuspend the pellet in one tenth of the starting volume of LB **[4]**.

2.2.1. Talent putting bacterial cultures in the incubator and closing the lid. **TEXT: Grow HT115 *E. coli* in LB with ampicillin (50 μg/mL)**

2.2.2. Talent putting the tubes with the bacteria in the centrifuge and closing the lid.

2.2.3. Talent aspirating the supernatant.

2.2.4. Talent resuspending the cell pellet.

2.3. Aliquot 200 microliters of concentrated bacteria to each plate **[1]** and allow the open plates to dry in a clean environment until all liquid has been absorbed **[2]**.

2.3.1. Talent adding bacteria to a few plates.

2.3.2. Plates in the laminar flow hood without a lid, drying.

3. Synchronization and Progeny Production

3.1. To synchronize *C. elegans* with hypochlorite treatment, wash gravid hermaphrodites twice with M9 buffer **[1]**. Then, transfer them in fresh tube and incubate in 5 milliliters of hypochlorite solution for 5 minutes **[2]**, shaking them every minute **[3]**. After the incubation, spin down the animals and wash them 3 times with M9 buffer **[4]**.

3.1.1. Talent washing animals with M9 buffer, with the buffer container in the shot.

3.1.1.1 Added shot: Talent transferring animals in fresh tube

3.1.2. Talent adding hypochlorite solution to the animals.

3.1.3. Talent shaking the tube with the worms.

3.1.4. Talent putting the worms in the centrifuge and closing the lid.

3.2. Allow embryos to hatch in tubes overnight in 3 milliliters of M9 solution with rotation at 20 degrees Celsius **[1]**.

3.2.1. Embryos rotating in the incubator.

3.3. Calculate the density of L1 animals by dropping 10 microliters of L1 solution 3 times onto a 6-centimeter plate **[1]** and counting the number of L1 animals **[2]**. Periodically mix the L1 solutions to prevent the animals from settling **[3]**.

3.3.1. Talent dropping solution on the plate.

3.3.2. Talent counting the animals.

3.3.3. Talent mixing the solution.

3.4. Seed 50 L1 animals onto each plate **[1]**, count and record the number seeded **[2]**, and move the plates to a 20-degree Celsius incubator **[3]**.

3.4.1. Talent seeding the worms on a plate.

3.4.2. Talent counting the animals and recording the number.

3.4.3. Talent putting the plates in the incubator and closing the door. *Videographer: Obtain multiple usable takes because this will be reused in 3.5.3.*

3.5. Alternatively, to synchronize animals via egg lay, place 5 to 10 young gravid adult animals onto each plate for 4 to 6 hours and allow them to lay eggs until there are approximately 50 eggs per plate **[1]**. Remove all gravid adults **[2]** and move the plates with the eggs to a 20-degree Celsius incubator **[3]**.

3.5.1. Talent placing animals on a plate and leaving it.

3.5.2. Talent removing the adults.

3.5.3. *Use 3.4.3.*

3.6. Grow the animals until L4 stage, which will take approximately 40 hours at 20 degrees Celsius **[1]**, then add 50 microliters of 160 X FUdR *(F-U-D-R)* **[2]**.

3.6.1. Talent taking plates out of the incubator.

3.6.2. Talent adding FUdR to plates.

4. Measuring Decline in Proteostasis

4.1. To measure decline in proteostasis in muscle tissue **[1]**, pick 20 animals and mount them on a microscope slide with a 3% agarose pad and a 5 microliter drop of 10 millimolar sodium azide **[2]**. *Videographer: This step is important!*

4.1.1. Talent setting up agarose pad on slide

4.1.2. Talent placing the animals on the agarose pad.

4.2. After all worms are immobilized, image the whole bodies of the animals using a 10 X magnification lens. Use a FITC or YFP filter and the same exposure for every animal **[1]**. When finished, discard the slides **[2]**.

4.2.1. Talent at the microscope, imaging the slides.

4.2.2. Talent discarding the slides. *Videographer: Obtain multiple usable takes because this will be reused in 4.5.2.*

4.3. Count the number of foci in the body wall muscles of the whole animal **[1]**. Foci are brighter punctuated signals that can be differentiated from the dimmer soluble signal in the background **[2]**. *Videographer: This step is important!*

4.3.1. Talent at the computer counting foci.

4.3.2. LAB MEDIA: Images for video production.pptx (slide #1)

4.4. On scoring days, look at the plates with the animals and record the number of paralyzed animals **[1]**, then remove paralyzed animals from the plate **[2]**. At the completion of the experiment, calculate the paralysis rate for each condition and plot the paralysis progression **[3]**.

4.4.1. SCOPE: Plate with paralyzed animals.

4.4.2. Talent removing a few paralyzed animals.

4.4.3. LAB MEDIA: Images for video production.pptx (slide #2)

4.5. To measure decline in proteostasis in neuronal tissue, mount the worms on a slide as previously described and take z-stack images of the head of the animals on a compound microscope using a 40x magnification lens **[1]**. Discard the slides after imaging **[2]**. *Videographer: This step is important!*

4.5.1. Show video of *z*-stack acquisition.

4.5.2. *Use 4.2.2.*

4.6. After acquiring the images, flatten the z-stacks and use them to quantify the number of foci in neurons located on the nerve ring area **[1]**. Plot the progression of YFP foci accumulation from days 4, 6, 8 and 10 **[2]**.

4.6.1. SCREEN: Z-stacks flattened and foci calculated. *Videographer: Film the screen.*

4.6.2. LAB MEDIA: Images for video production.pptx (slide #3)

4.7. On Day 2 of adulthood, pick 10 synchronized animals from the plate and place them on a 10 microliter drop of M9 buffer on a microscope slide. Repeat this step at least four times to get a sample of 40 or more animals **[1]**.

4.7.1. Talent putting animals on slide.

4.8. Video record the movement of the animals **[1]** for a period of 30 seconds on a stereomicroscope with a video-capable camera. Once all the videos with the animals

to be analyzed are recorded, play the video and score the body bends of each animal **[2]**. *Videographer: This step is important!*

4.8.1. Talent at the computer, looking at the movie and scoring the bends.

4.8.2. LAB MEDIA: worm body bends- unedited scope capture.mp4. *Video Editor: Use the author-edited body bend example and counting draft1.mp4 as a reference for editing this clip. Emphasize the body bends on one worm and show the bends being counted.*

4.9. Plot the number of body bends for each animal in a column graph where each dot represents the number of body bends in 30 seconds on the Y axis and the different conditions tested on the X axis **[1]**.

4.9.1. LAB MEDIA: Figure 2 C.

Results

5. Results: HPK-1 Promotes Proteostasis

5.1. The polyglutamine repeat model has been instrumental for the identification of genes that regulate the proteostatic network **[1]**. Muscle-specific poly-Q-YFP expression results in accumulation of fluorescent foci that are easy to quantify under a simple fluorescent dissecting microscope **[2]**.

5.1.1. LAB MEDIA: Figure 1 A.

5.1.2. LAB MEDIA: Figure 1 A, just the "Day 4 old" image, and B. *Video Editor: In the "Day 4 old" image, emphasize the areas that the red arrows are pointing to.*

5.2. The animals become paralyzed during mid-life as the proteome within the muscle collapses due to the proteotoxic effect of the reporter **[1]**. The age-associated decline in neuronal proteostasis can be followed by directly quantifying aggregate formation **[2]** and declines in coordinated body-bends after placing animals into liquid **[3]**.

5.2.1. LAB MEDIA: Figure 1 C.

5.2.2. LAB MEDIA: Figure 2 A, just the fluorescence images (bottom two) and B.

5.2.3. LAB MEDIA: Figure 2 C.

5.3. This method has been used to show that the homeodomain interacting protein kinase, a transcriptional cofactor, influences proteostasis during aging by regulating expression of autophagy and molecular chaperones **[1-TXT]**.

5.3.1. LAB MEDIA: Figure 3 A – C. *Video Editor: Label A "***Control***", B "***hpk-1* **RNAi***", and C "***hpk-1* **Overexpressing***".*

5.4. The loss of *hpk-1* increases the number of Q35-YFP aggregates that accumulate during aging **[1]**. Control animals displayed an average of 18 aggregates **[2]** while the *hpk-1* null mutant and *hpk-1* RNAi-treated animals displayed an average of 28 and 26 aggregates, respectively **[3]**.

5.4.1. LAB MEDIA: Figure 3 D.

5.4.2. LAB MEDIA: Figure 3 D. *Video Editor: Emphasize the black circles.*

5.4.3. LAB MEDIA: Figure 3 D. *Video Editor: Emphasize the white circles and white squares.*

5.5. By day 8 of adulthood, 77 to 78% of *hpk-1* deficient animals were paralyzed **[1]**, compared to only 50% of the control **[2]**. Additionally, overexpression of *hpk-1* was demonstrated to regulate protein aggregate formation and protect aging animals from Q35-YFP-associated paralysis during aging **[3]**.

5.5.1. LAB MEDIA: Figure 3 E. *Video Editor: Emphasize the white circles and white squares.*

5.5.2. LAB MEDIA: Figure 3 E. *Video Editor: Emphasize the black circles.*

5.5.3. LAB MEDIA: Figure 3 C and E. *Video Editor: Emphasize the white triangles on E.*

Conclusion

6. Conclusion Interview Statements

6.1. **Maria Lazaro-Pena:** This method measures general decline of the proteome within a cell-type. Many methods exist to assess changes in specific components of the proteostatic network. Together they provide a comprehensive picture.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 4.7.1*

6.2. **Andrew Samuelson:** Declining proteostasis is a hallmark of aging. This approach allows researchers to quantify this decline. When combined with genetic analysis, it is a powerful tool for discovery.

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