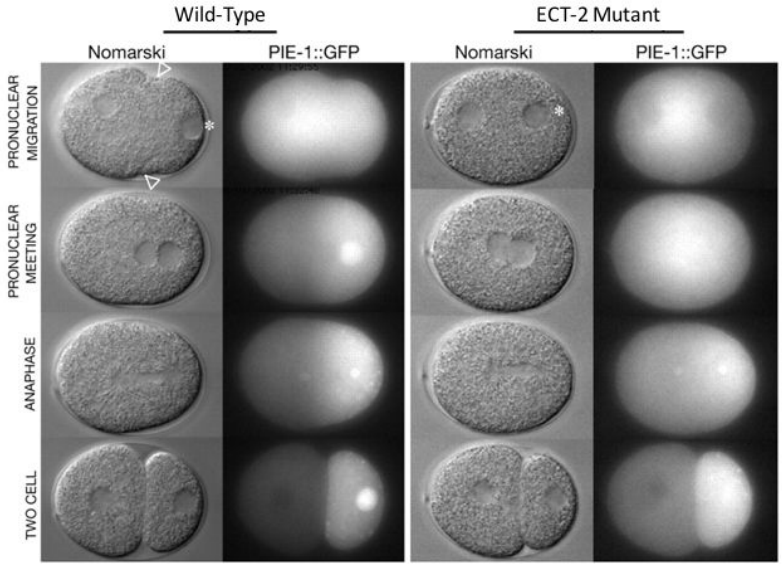
Educator Resources By Kanaga Rajan
Seminar: How to Create a Body Axis
Speaker: Geraldine Seydoux

1. For the following question, choose the best answer. Which of the following is true about body-axis establishment in *C.elegans*:
    a. The zygote is polarized as early as the two-cell stage
    b. PAR proteins are important for embryo polarization
    c. Cytoplasmic protein polarization is important for body axis formation
    d. a and b only
    e. All of the above

2. How does fertilization by the sperm regulates polarization and establishes the anterior-posterior body axis in *C.elegans*.

Use the following information to answer question #3:
In *C.elegans*, ECT-2 and PIE-1 are other proteins important for the establishment of body axis. In the following experiment, researchers created a ECT-2 mutant and observed localization of PIE-1 (via a GFP-tagged protein) in *C.elegans* at the one and two-cell stages. In the data below, the anterior-posterior orientation is left-right, respectively.



(Zonies et al. Development 2010)

3. For the following question, choose the best answer. Based on the information above, which of the following are true:
    a. Mutant ECT-2 does not polarize correctly
    b. ECT-2 is necessary for proper PIE-1 polarization to the posterior pole
    c. ECT-2 mutation triggers PIE-1 localization to the anterior pole
    d. A and C only
    e. None of the above

4. For the following question, choose the best answer. Which of the following is true about PAR proteins in *C.elegans*:
    a. There are 2 anterior PAR proteins
    b. There are 2 posterior PAR proteins
    c. PAR-2 is a kinase
    d. A and B only
    e. All of the above

5. True or False: Pre-polarization, anterior PARs prevent posterior PARs form binding to the membrane.

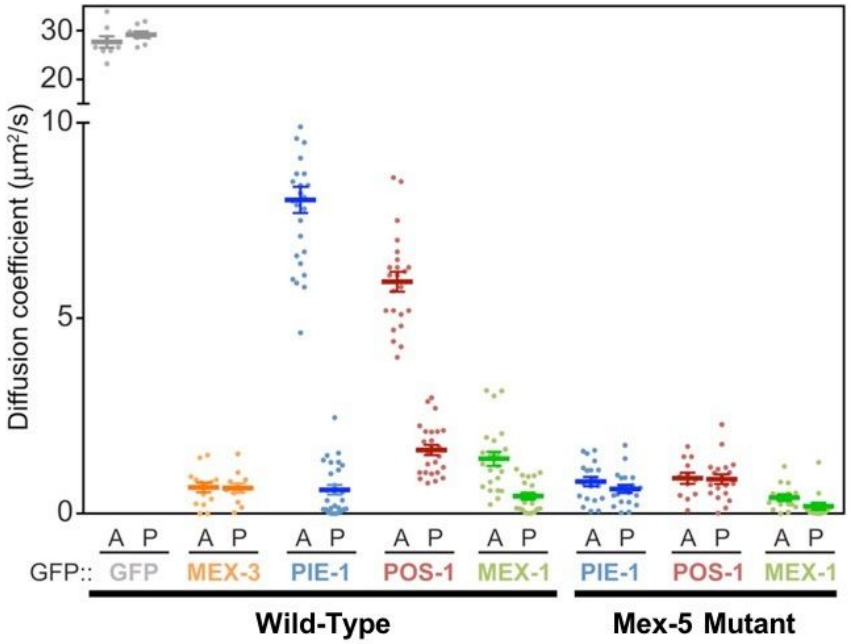
6. For the following question, choose the best answer. Which of the following is true about MEX-5 and its role in *C.elegan* body-axis establishment:
    a. MEX -5 is directly phosphorylated by PAR-3
    b. MEX-5 establishes a protein gradient across the embryo
    c. MEX-5 is localized in the cytoplasm
    d. a and b
    e. b and c

7. Briefly explain what is unique about MEX-5 polarization?

8. Scientists measured the diffusion Protein W, X, Y and Z and calculated the diffusion coefficients ($\mu m^2/s$) to be 50, 17, 5, and 34, respectively.
    a. Please list Protein W, X, Y and Z from slowest to fastest diffusion.

    b. Given what you learned from Mex-5, what can you infer about the size of these complexes? Briefly explain.

Use the following information to answer question #9:
PIE-1, POS-1 and MEX-1 are all proteins important for *C.elegan* development and establish gradients in the embryo cytoplasm. Seydoux's lab wanted to dissect the role of these proteins in establishing the anterior-posterior body axis, specifically in relation to MEX-5. Researchers therefore knocked down MEX-5 using RNAi and measured the diffusion and diffusion coefficients for each of these proteins (Note: A = Anterior and Posterior).

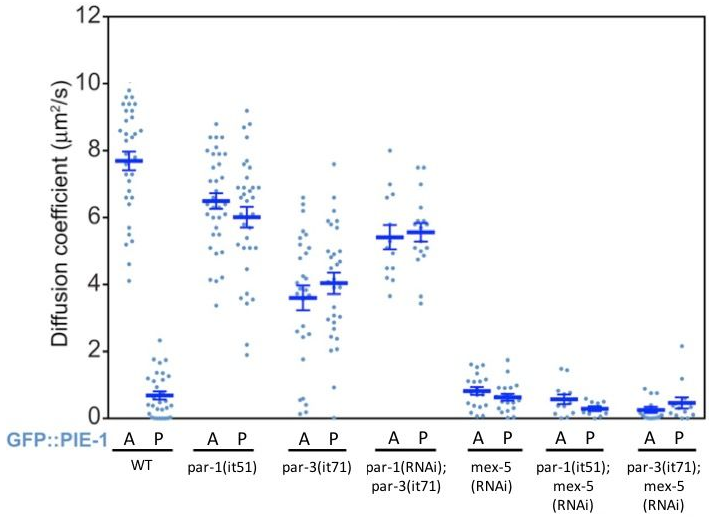


(Modified from Wu et al. MBoC 2015)

9. Answer the following questions based on the data above:
    a. Compare and contrast the expressions pattern of PIE-1 and MEX-1 in wild-type animals and how can you tell.

    b. How does PIE-1, POS-1 and MEX-1 expression patterns change when MEX-5 expression is disrupted?

Use the following information to answer question #10:
RNAi was used to disrupt mex-5 expression in PAR-1 and PAR-3 mutant backgrounds or in combination with par-1 (RNAi). Diffusion coefficients for a GFP-tagged PIE-1 were determined. using fluorescence correlation spectroscopy (FCS). par-1(it51) and par-3(it71) are mutants of PAR-1 and PAR-3.



(Modified from Wu et al. MBoC 2015)

10. For the following question, choose the best answer.
    a. Posterior PIE-1 moves faster in par-1(it51) mutant than WT
    b. Posterior PIE-1 moves faster in par-3(it71) mutant than WT
    c. Anterior PIE-1 moves slower in mex-5(RNAi) than WT
    d. All of the above
    e. None of the above

Educator Resources (ANSWERS):
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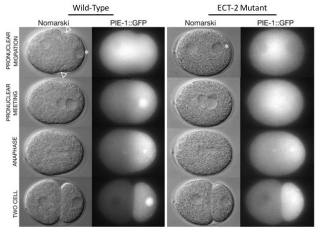
1. For the following question, choose the best answer. Which of the following is true about body-axis establishment in *C.elegans*:
    a. The zygote is polarized as early as the two-cell stage
    b. PAR proteins are important for embryo polarization
    c. Cytoplasmic protein polarization is important for body axis formation
    d. a and b only
    e. All of the above

2. How does fertilization by the sperm regulates polarization and establishes the anterior-posterior body axis in *C.elegans*.
    - **Sperm entrance determines the posterior site. Before fertilization, the anterior complex (Par-3, Par-6 & PKC-3) prevents the posterior complex (Par-2 & Part-1) from binding the membrane. Note: PKC-3 phosphorylates Par-2, which prevents its binding to the membrane.**
    - **Upon fertilization, the sperm brings microtubules which prevents PKC-3 phosphorylation of Par-2. Now Par-2 is able to bind to the membrane and recruit Par-1.**
    - **Par-1 phosphorylates adjacent Par-3, which, upon phosphorylation, can no longer bind the membrane. This releases the anterior complex from the membrane in the posterior side (where microtubules localize, protecting Par-2).**
    - **Also, after fertilization, through a mechanism not completely understood, the cytoskeleton of the embryo reorganizes, and localizes the anterior complex in the anterior side.**

    Use the following information to answer question #3:
    In *C.elegans*, ECT-2 and PIE-1 are other proteins important for the establishment of body axis. In the following experiment, researchers created a ECT-2 mutant and observed localization of PIE-1 (via a GFP-tagged protein) in *C.elegans* at the one and two-cell stages. In the data below, the anterior-posterior orientation is left-right, respectively.



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3. For the following question, choose the best answer. Based on the data above, which of the following is true:
    a. Mutant ECT-2 does not polarize correctly **(FALSE - cannot tell by this figure)**
    b. ECT-2 is necessary for proper PIE-1 polarization to the posterior pole
    c. ECT-2 mutation triggers PIE-1 localization to the anterior pole (**FALSE**)
    d. a and c only
    e. None of the above

4. For the following question, choose the best answer. Which of the following is true about PAR proteins in *C.elegans*:
    a. There are 2 anterior PAR proteins **(FALSE - 3 anterior PARs - PAR-3, PAR-6 and PKC-3)**
    b. There are 2 posterior PAR proteins
    c. PAR-2 is a kinase **(FALSE - PAR-1 is a kinase)**
    d. a and b only
    e. All of the above

5. True or False: Pre-polarization, anterior PARs prevent posterior PARs form binding to the membrane. **TRUE**

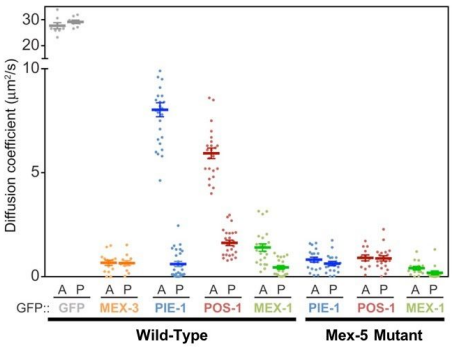
6. For the following question, choose the best answer. Which of the following is true about MEX-5 and its role in *C.elegans* body-axis establishment:
    a. MEX -5 is directly phosphorylated by PAR-3
    b. MEX-5 establishes a protein gradient across the embryo
    c. MEX-5 is localized in the cytoplasm
    d. a and b
    e. **b and c**

7. Briefly explain what is unique about MEX-5 polarization?
    The MEX-5 protein gradient is created entirely by protein <u>diffusion</u>. (After being phosphorylated by PAR-1, the smaller MEX-5 species moves faster and moves to the anterior end of the embryo. Once there, due to the lack of PAR-1 at the anterior pole, MEX-5 phosphorylation is lost and MEX-5 shifts back to the larger species which moves more slowly.)

8. Scientists measured the diffusion Protein W, X, Y and Z and calculated the diffusion coefficients ($\mu m^2/s$) to be 50, 17, 5, and 34, respectively.
    a. Please list Protein W, X, Y and Z from slowest to fastest diffusion.
    Slowest - Y (5), X (17), Z (34), W (50) - Fastest

b. Given what you learned from Mex-5, what can you infer about the size of these complexes? Briefly explain.
ANSWER: The fast complex (W) is likely to be smaller than the slower complex (Y).

Use the following information to answer question #9:
PIE-1, POS-1 and MEX-1 are all proteins important for *C.elegan* development and establish gradients in the embryo cytoplasm. Seydoux's lab wanted to dissect the role of these proteins in establishing the anterior-posterior body axis, specifically in relation to MEX-5. Researchers therefore knocked down MEX-5 using RNAi and measured the diffusion and diffusion coefficients for each of these proteins (Note: A = Anterior and Posterior).



(Modified from Wu et al. MBoC 2015)

9. Answer the following questions based on the information above:
   a. Compare and contrast the expressions pattern of PIE-1 and MEX-1 in wild-type animals and how can you tell.
   PIE-1 - makes a protein gradient from posterior to anterior, with highest concentration of PIE-1 in posterior. The species in the posterior has lower/no diffusion coefficient so they do not move while PIE-1 in the anterior moves, and can therefore move from anterior to middle/posterior. On the other hand, MEX-1 exhibits weak/no expression gradient. Due to lack of diffusion in posterior and weak diffusion in anterior, MEX-1 is more or less expressed equally throughout the embryo (highly in the posterior and slightly less in anterior)

   Both proteins are more strongly expressed in the posterior and both proteins have a higher diffusion coefficient in the anterior (suggests greater diffusion movement) and lower coefficient in the posterior (suggests little to no movement)

b. How does PIE-1, POS-1 and MEX-1 expression patterns change when MEX-5 expression is disrupted?
Loss of MEX-5 disrupts diffusion of all three proteins in the anterior (low to no diffusion). PIE-1, POS-1 and MEX-1 are localized more or less evenly at both poles of the zygote. MEX-1 is expressed at the posterior and anterior most ends.

Use the following information to answer question #10:
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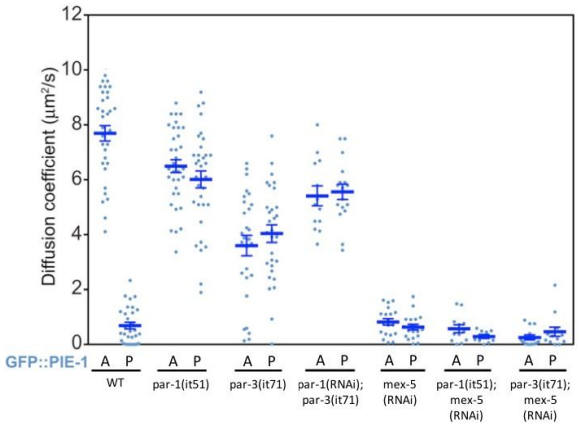

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    c. Anterior PIE-1 moves slower in mex-5(RNAi) than WT
    d. All of the above
    e. None of the above