Understanding Microtubule Dependent Signaling in the generation of Cellular Asymmetries

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Aim of the investigation

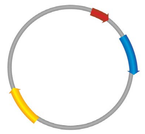
Polarity is displayed by most complex beings in the form of structural asymmetries within physiological systems and cells. The earliest polarity is seen in C. Elegans is in the single-cell embryo. **Our work is aimed at investigating how polarity is established and maintained in this stage of development.**

Our Model

- In order to determine whether a gene is responsible for loss of polarity through the Microtubule (MT) pathway, the NOP-1 mutant, which presents a loss of the actomyosin meshwork pathway, was used. The gene of interest would then have been knocked out through RNAi in order to assess its involvement in the surviving pathway. If this were the case, the gene knockdown would result in polarity loss.

Research outline

Step 1



Knockdown genes thought to be involved in MT pathway through RNAi

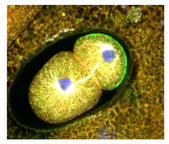
Step 2

Perform embryonic lethality screens for said genes

Step 3

Re-test genes with non significant-results by changes in RNAi exposure and larval stage

Step 4



Perform confocal IF to characterize phenotype of knockdown and quantify polarity loss

Introduction

In C. Elegans, polarity is established through two pathways:

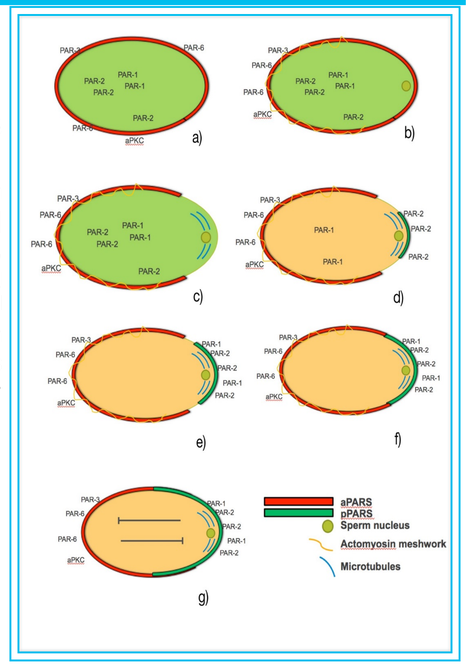
Actomyosin Meshwork (AMM) & aPARs (aPKC3, PAR3, PAR6):

Prior to symmetry breaking, anterior PAR proteins (aPARs) are distributed uniformly at the cortex [a]. Sperm entry signals retract from the cortex nearest the centrosome, allowing pPAR accumulation [c]. [1] aPARs retraction occurs through "**cortical flows**", a network of interconnected **actomyosin** foci and cables under the plasma membrane which begins after completion of the meiotic divisions [b-f]. [2]

This network disperses at the point of contact as the paternally derived centrosome reaches the cortex. [3] Vanishing of aPARs from the cortex nearest the centrosome suggests that cortical flows carry the aPARs. This displacement by the cortical flows allows pPARs loading [d]. [2] pPARs can also access the posterior cortex in the absence of cortical flows, as seen with the NOP-1 mutant, allowing the domains to be established, more slowly. [4;5;6]

Sperm centrosome-enucleated microtubules (MT) & pPARs (PAR-2, PAR-1, LGL-1):

Loading and positioning of **PAR-2** is associated with microtubule nucleation by the sperm centrosome [d] [7;8;9]. PAR-2 loads first and recruits PAR-1[e], which, in turn, can phosphorylate PAR-3 (aPAR), triggering exclusion of aPARS from the posterior domain.[10] Before symmetry breaking, PAR-2 is maintained in the cytosol through phosphorylation by aPKC-3. It is thought that microtubules compete with aPKC-3 for access to PAR-2. [g] [3]



Results

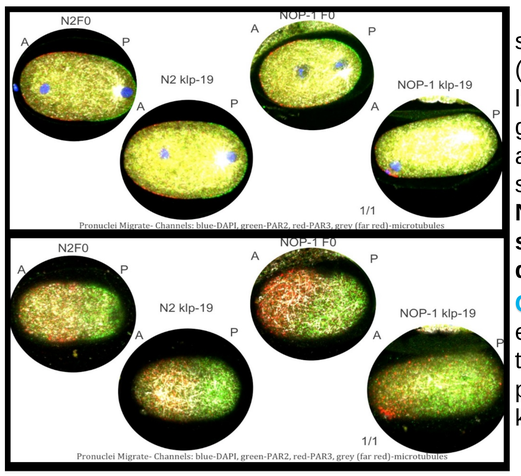
Screen Analysis

Gene + condition	Enhancer by ε at 28h?	Enhancer by ε at 52h?	IF?
gpb-1 (L4 10%)			Y
B0491.5 (L4 50%)			N
Y65B4BR.5 (L4 100%			Y
paa-1 (L4 10%)			N
let-754 (L4 100%)			Y
plk-1 (L4 10%)			N
klp-19 (L4 100%)			N
kin-3 (L4 100%)			Y
ril-1 (L4 100%)			N
cdc-25 (L4 100%)			N
arf-1.2 (L4 100%)			Y
rack-1 (L3 100%)			Y
ego-2 (L3 100%)			N
cnt-2 (L4)			Y

Gene + condition	Enhancer by ε at 28h?	Enhancer by ε at 52h?	IF?
paa-1 (YA 100%)			N
plk-1 (YA 100%)			N
klp-19 (L4 10%)			Y
kin-3 (L4 50%)			N
cdc-25 (L4 10%)			N

Repeats

- The table shows the results of the embryonic lethality screens. Each gene was tested in triplicate. The green squares indicate significant positive hits – i.e. the eggs exposed to the RNAi hatched less in the NOP-1 mutant than in the N2 wild type.



Midplain section of embryos stained for PAR-3 (red) PAR-2 (green) and DAPI (blue). From left to right: N2 (wt) without any gene RNAi (F0), N2 with klp-19 and NOP-1 without RNAi (F0) shows established polarity; **NOP-1 with klp-19 RNAi shows loss of polarity domains.**

Cortical view of the same embryos as those above taken to show more clearly the loss of polarity domains in the NOP-1 klp-19 embryo.

Conclusion

- The screens resulted in 8 candidate genes (gbp-1, Y65B4BR.5, let-754, klp-19, arf-1.2, rack-1, ego-2, cnt-2) for a qualitative investigation of the phenotype resulting from their knockdown.
- klp-19 and gbp-1 were analysed through confocal immunofluorescence. Only one embryo out of the 10 analysed for klp-19 showed polarity loss, and none for gbp-1(RNAi), contrasting with the significant results obtained from the embryonic lethality enhancement screen.

Acknowledgments

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Works Cited

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