Investigating the role of a Rac homolog in mitotic spindle positioning during asymmetric division in *C. elegans* embryos

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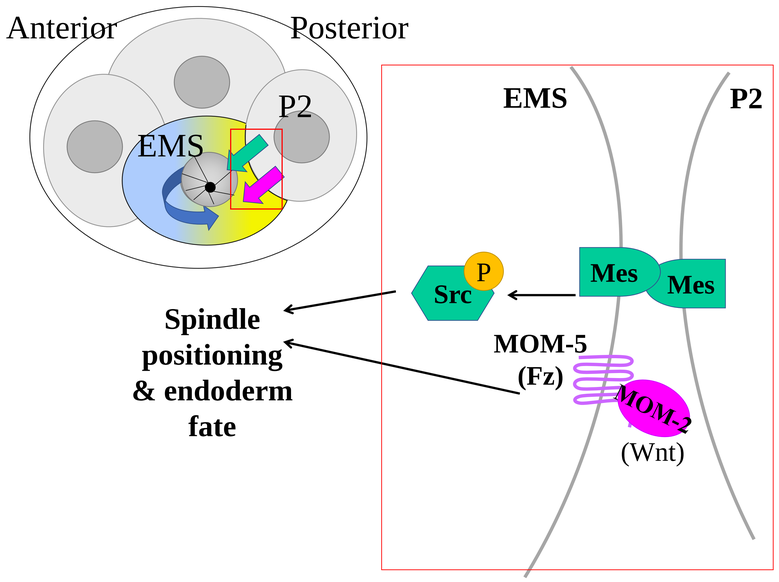
Abstract

Asymmetric cell division is important for generating cell type diversity during development and for tissue homeostasis. Cellular asymmetry can be generated either by internal or external cues. In many systems, pulling forces generated by a complex of dynein, LIN-5/Mud/NuMA, and the heterotrimeric G-protein Gαi orient the spindle along the anterior/posterior axis. In the one-cell embryo of Caenorhabditis elegans, the complex is asymmetrically localized by the G-protein regulator LET-99 in response to internal polarity cues. In contrast to the one-cell, the EMS cell divides asymmetrically in response to signals from the neighboring cell, P2. In this division, partially redundant Wnt and MES-1/SRC-1 pathways specify endoderm versus mesoderm fate and instruct the EMS mitotic spindle to orient along the anterior/posterior axis prior to division. We previously identified LET-99 and the dynein regulator LIN-5/Mud/NuMA as regulators of EMS spindle orientation, suggesting that some of the components of the one-cell force-generating complex are also at work in the EMS cell. We have now identified CED-10, a homolog of the small G protein Rac1, as a member of the MES-1/SRC-1 pathway. We hypothesize that MES-1/SRC-1 signaling regulates an antagonistic interaction between LET-99 and CED-10 to tune cortical branched actin levels and thereby regulate the localization of force generators. To test whether CED-10 acts upstream or downstream of SRC-1 activity, we stained for SRC-dependent phosphorylation at the P2-EMS contact. Preliminary results show that phosphorylation signal intensity is not significantly different between wild-type and ced-10 mutant embryos. To determine whether branched actin is part of the MES-1/SRC-1 pathway, we depleted the actin nucleator Arp-2/3 by RNAi in a Wnt mutant background. Unexpectedly, we found that branched actin and Wnt signaling are required together during the earlier spindle orientation of the P1 cell, which is the precursor to both EMS and P2. We are currently generating strains to test whether P1 polarity is perturbed in arp-2/3;wnt embryos. We are also testing whether LET-99 affects CED-10 localization. Overall, this work will contribute to a more complete understanding of cytoskeletal regulation by cell-cell signaling during asymmetric cell division.

Introduction

- Asymmetric cell division generates cell type diversity
- Asymmetric division requires a cue to orient cell polarity, align the mitotic spindle with it, and establish daughter cell fate
- The EMS (Endoderm and MeSoderm progenitor) cell of the *Caenorhabitis elegans* 4-cell embryo is an example of externally-cued polarity



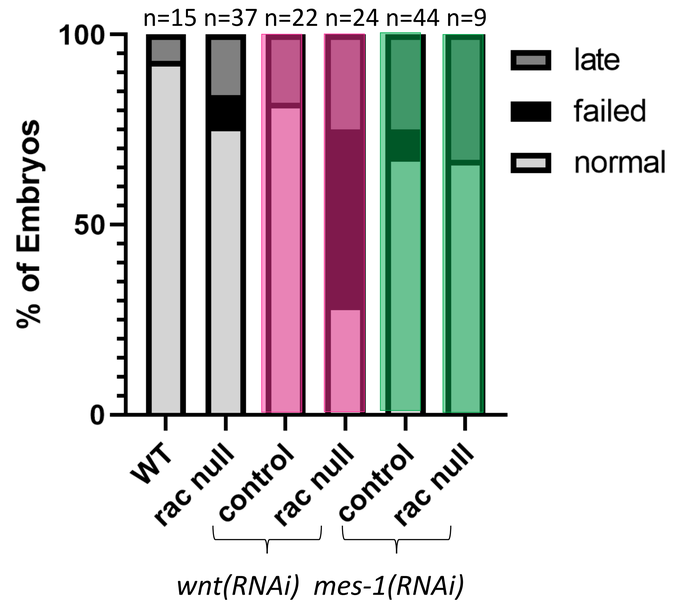
- The P2 cell provides a cue through two partially-redundant signaling pathways – Wnt/Frizzled and MES-1/SRC-1 – to orient the EMS spindle and establish endoderm fate in the E daughter cell
- EMS spindle positioning is thought to require a conserved force-generating complex containing dynein and adaptor LIN-5 (Mud, NuMA)

Objective: Identify additional members of the MES-1/SRC-1 pathway and determine how the pathway regulates EMS spindle orientation

Results

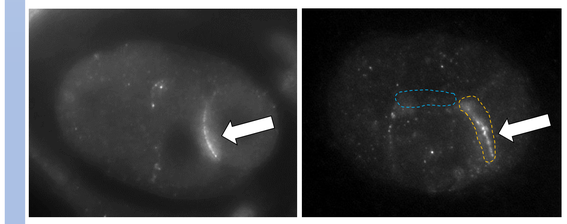
Rac contributes to EMS spindle positioning in the MES-1/SRC-1 pathway and not the Wnt pathway

The Rac homolog CED-10 (hereafter "Rac") was examined during EMS spindle positioning based on its known role in a later Wnt dependent asymmetric division. Scoring EMS spindle rotation events showed that loss of Rac enhances the defect seen in *wnt* mutants but not *mes-1* mutants.



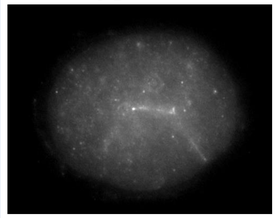
Rac acts upstream or at the level of SRC-1 activation

Anti-phosphotyrosine staining showed that SRC-1 activation levels in *rac* mutants are between those of WT embryos and *mes-1* mutants. This result indicates a role for Rac upstream of SRC-1 target phosphorylation.



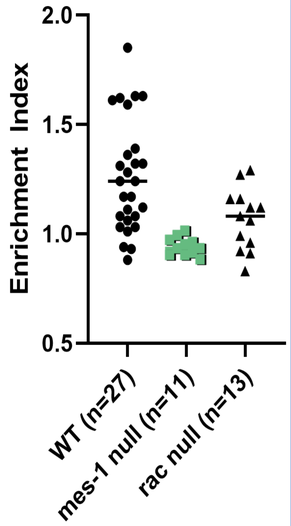
Wild-type

rac null mutant



mes-1 null mutant

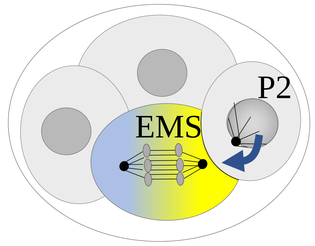
Enrichment Index = EMS/P2 contact : EMS/ABp contact

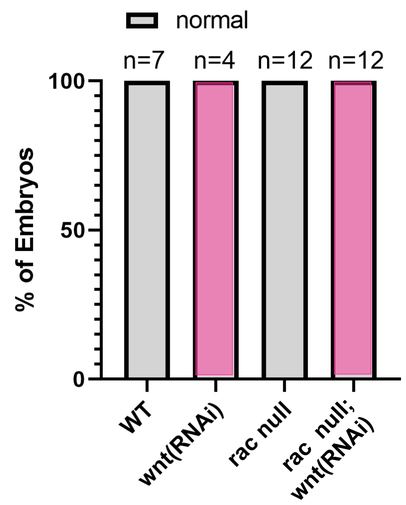


Results, continued

Rac does not affect P2 spindle orientation

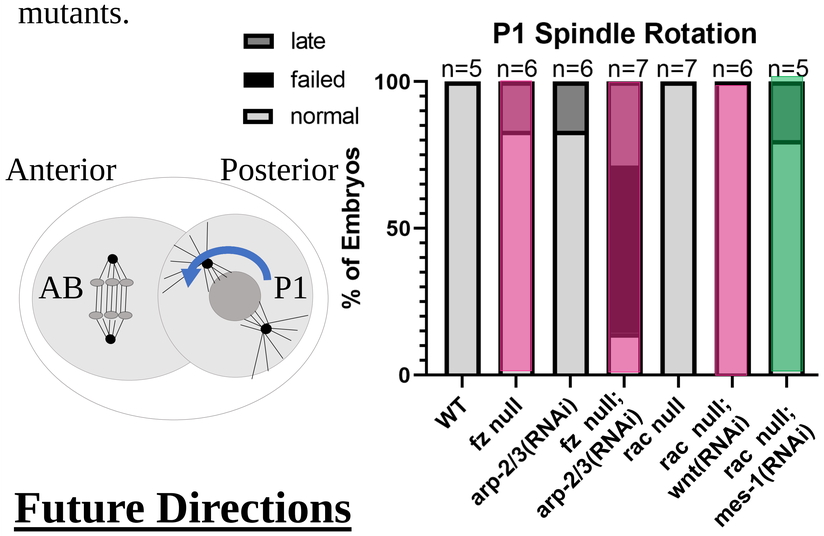
MES-1 signaling is also required for P2 spindle orientation. However, loss of Rac does not affect the P2 spindle, suggesting that Rac acts only in EMS.





Branched actin and Frizzled are required together for P1 spindle orientation

To test if branched actin (a known target of Rac) plays a role in EMS spindle positioning, we depleted branched actin in a *frizzled* mutant background. Unexpectedly, we observed a high occurrence of failed spindle rotations in the earlier P1 cell division. This defect was not observed in *rac* or *rac;wnt* mutants.



Future Directions

- Test whether branched actin and Frizzled are required for PAR polarity at the two-cell (or one-cell) stage
- Test whether Rac-dependent actin is required for P1 orientation
- Test whether Rac affects PAR polarity in P2
- Test whether Rac affects endoderm fate specification
- Test whether Rac affects force-generator localization