

CDC-42 encodes dynamically stable asymmetries in the *C. elegans* zygote via an incoherent feed-forward loop

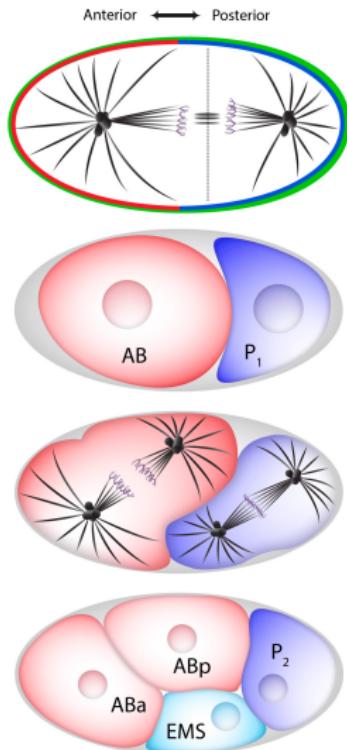
Ondrej Maxian, Cassandra Azeredo-Tseng, Ed Munro & Others

Munro Lab Group Meeting
April 15, 2024

Cell polarization

Spatial differences in protein concentration

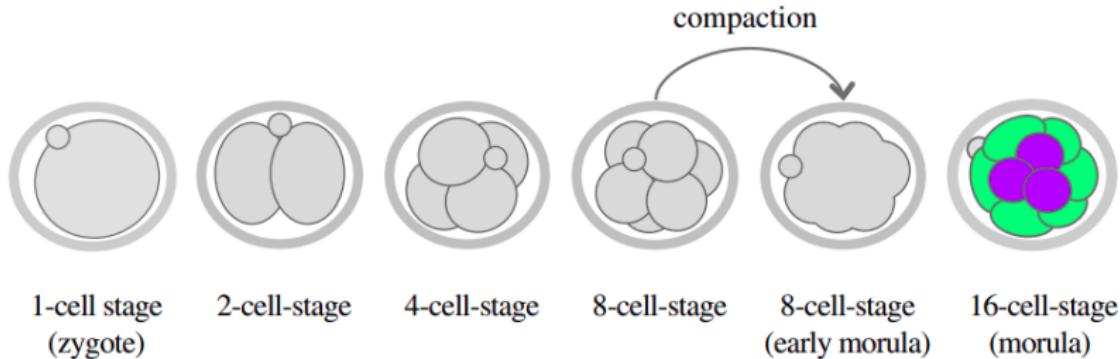
- ▶ Encode cell fate decisions
- ▶ Vital for proper development



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One-cell *C. elegans* model system

Ingredients

- ▶ PAR proteins
 - ▶ aPARs (PAR-3, PAR-6/PKC-3, CDC-42)
 - ▶ pPARs (PAR-1, PAR-2, CHIN-1)
- ▶ Actomyosin flows

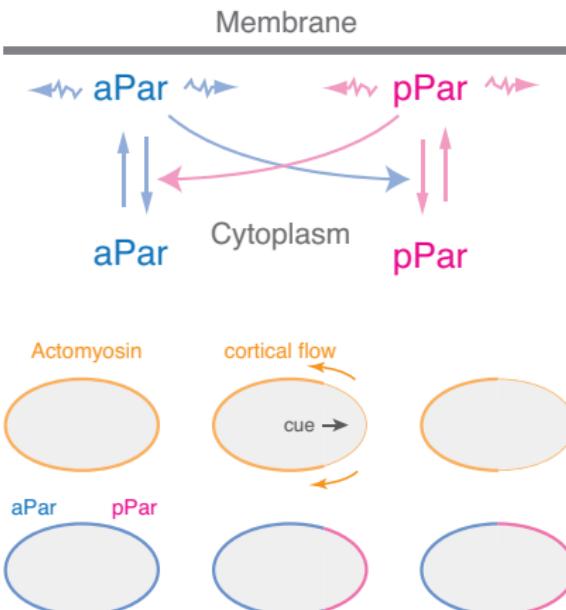
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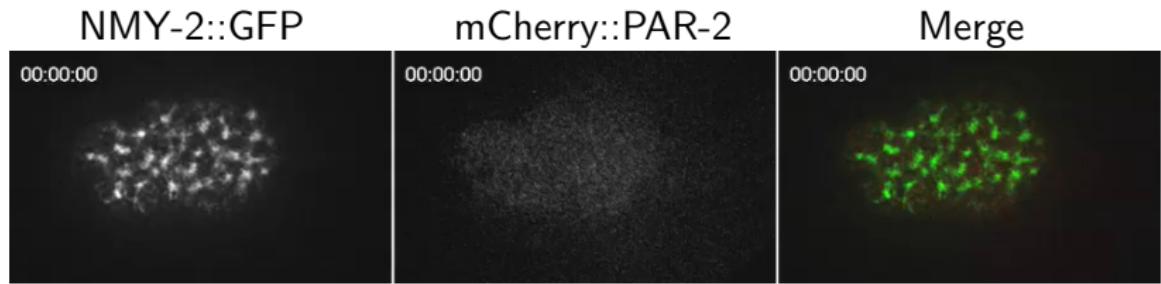
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Wild type sequence

- ▶ Centrosomes → PAR-2 localized
- ▶ Sperm cue → Myosin inhibition
- ▶ Expansion of boundary to stable point (“establishment”)
- ▶ “Maintenance:” boundary stays



Movie: *C. elegans* wild type

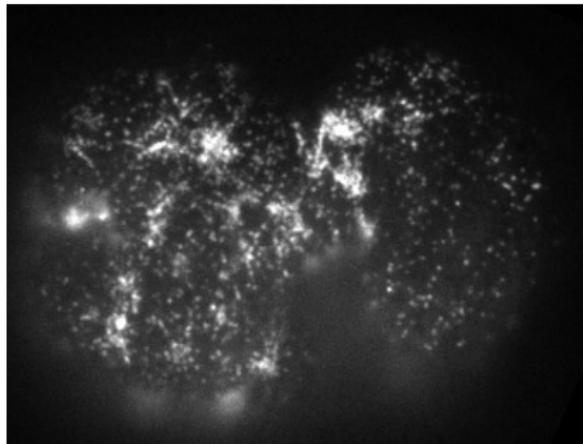


Standard version of the story

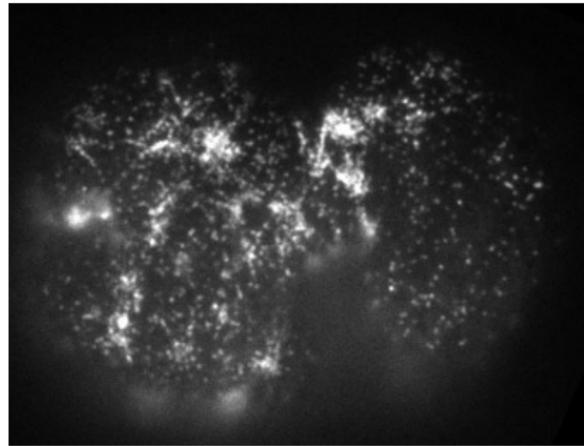
No flows in maintenance?

Gross paper

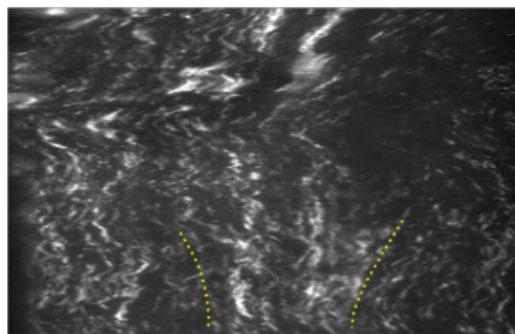
Maintenance phase: a closer look



Maintenance phase: a closer look

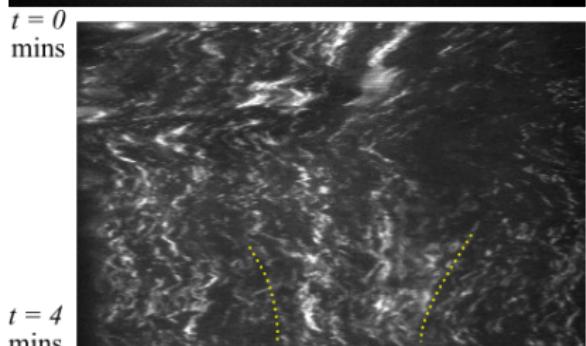
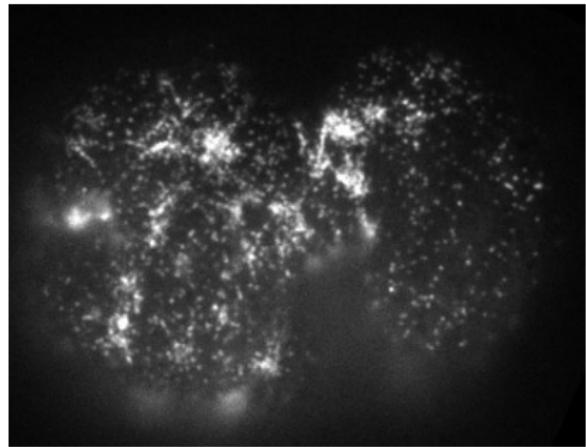


$t = 0$
mins

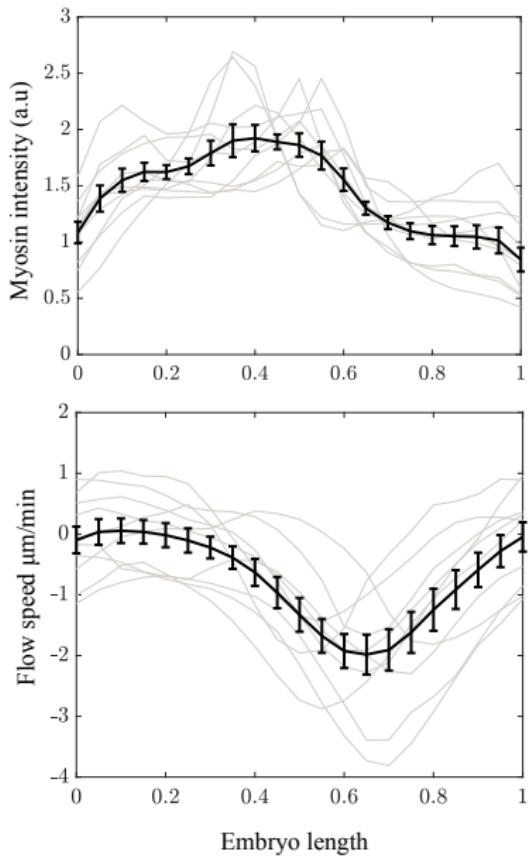


$t = 4$
mins

Maintenance phase: a closer look



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mins



Questions to answer

Late maintenance: more myosin on anterior, steady A-directed flow

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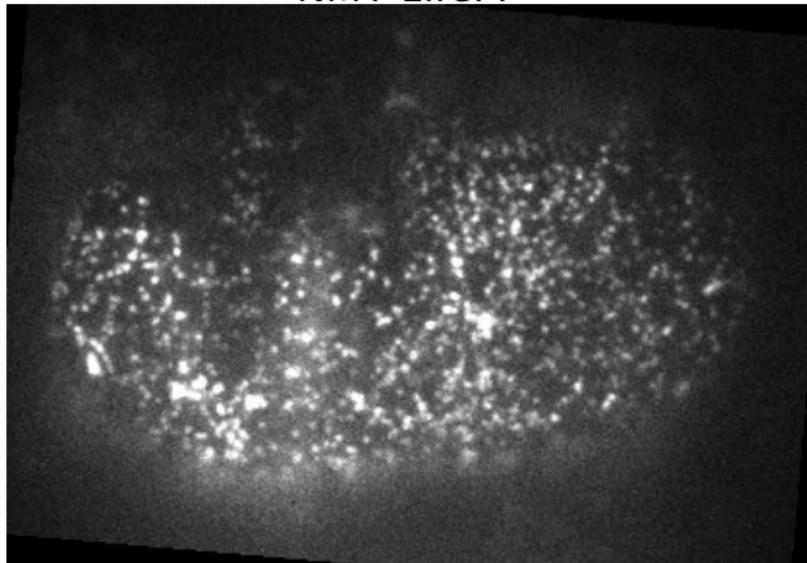
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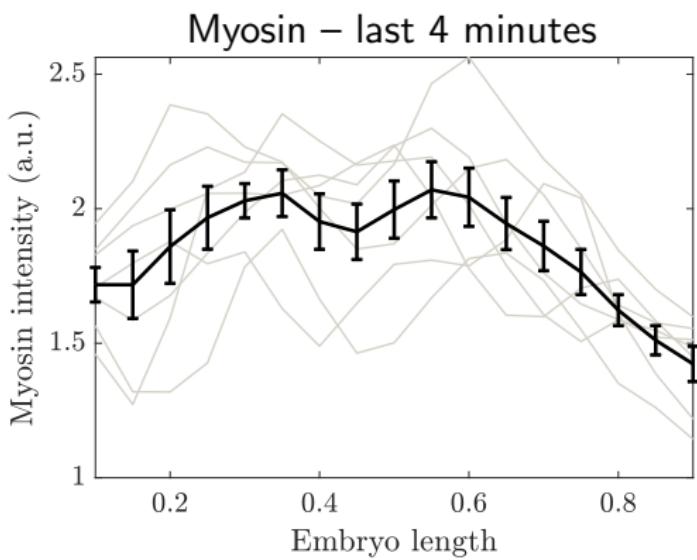
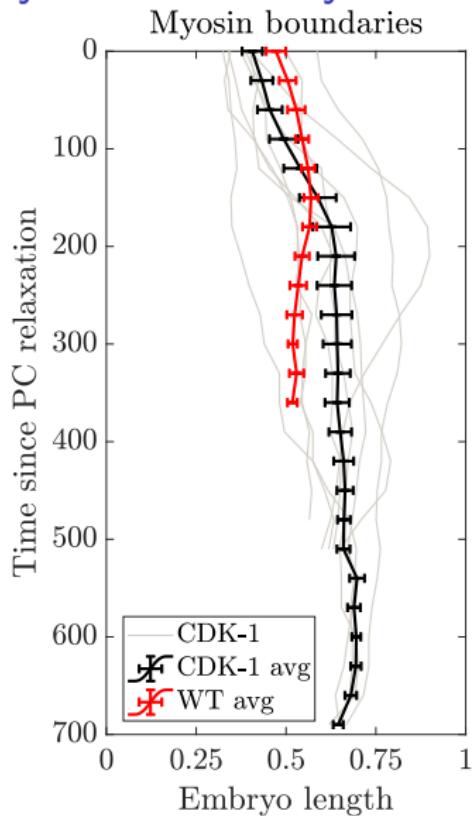
Late maintenance is (roughly) a steady state

Use CDK-1 (RNAi) to expand maintenance phase; boundary actually moves towards posterior

NMY-2::GFP

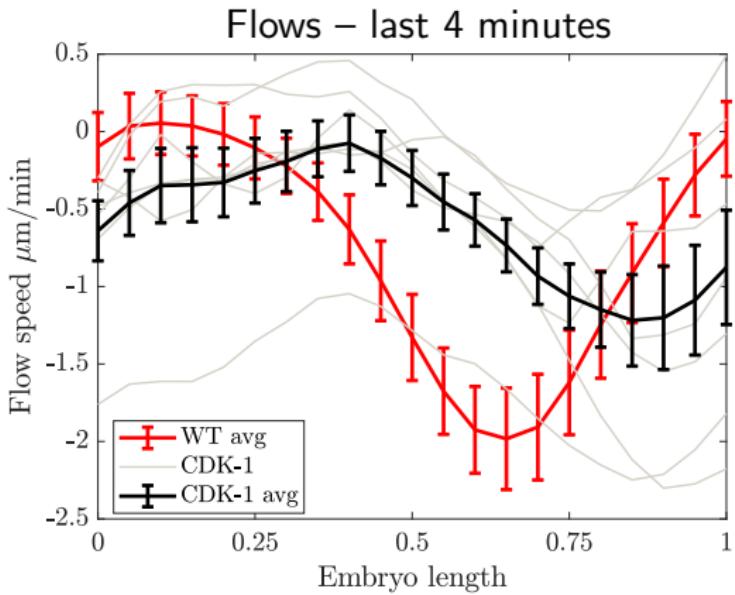
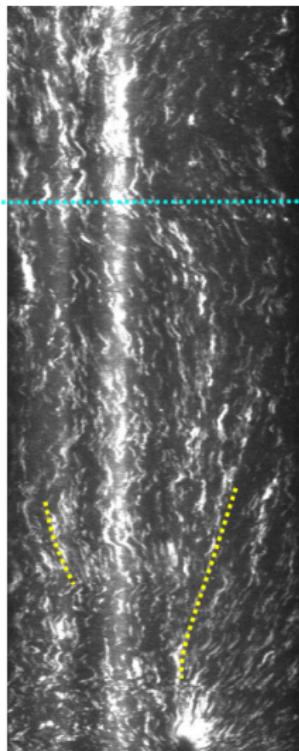


Myosin boundary stable



Slight shift towards posterior; but still have A/P asymmetry

Steady flow profile in CDK-1 embryos



Still see contraction into medial domain (nonzero flows)

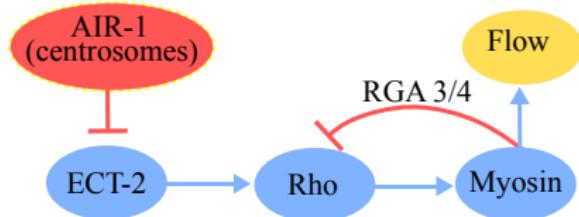
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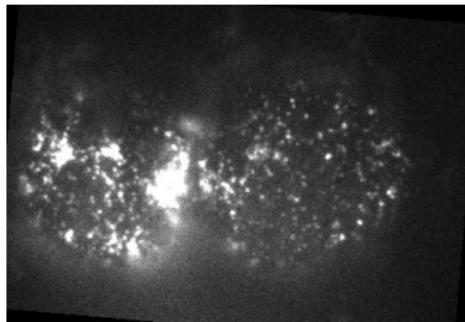
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Distinction between establishment and maintenance

Establishment phase

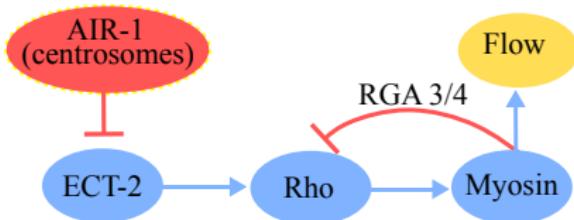


- ▶ Pulsatile contractility
(Michaux et al., 2018)
- ▶ Governed by rho

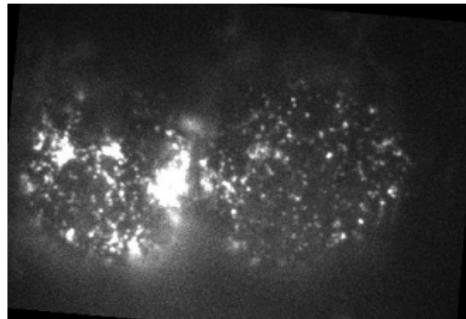


Distinction between establishment and maintenance

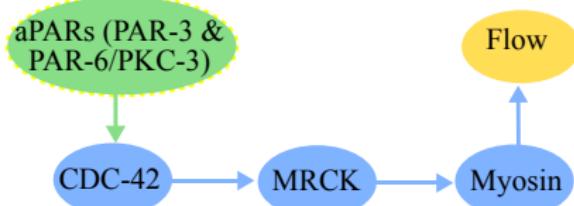
Establishment phase



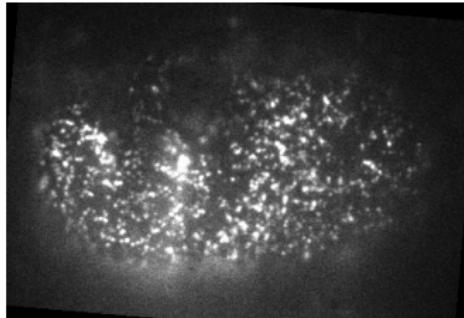
- ▶ Pulsatile contractility
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Maintenance phase



- ▶ Diffuse myosin clusters
- ▶ Governed by CDC-42
(through MRCK)



Starting polarity from maintenance

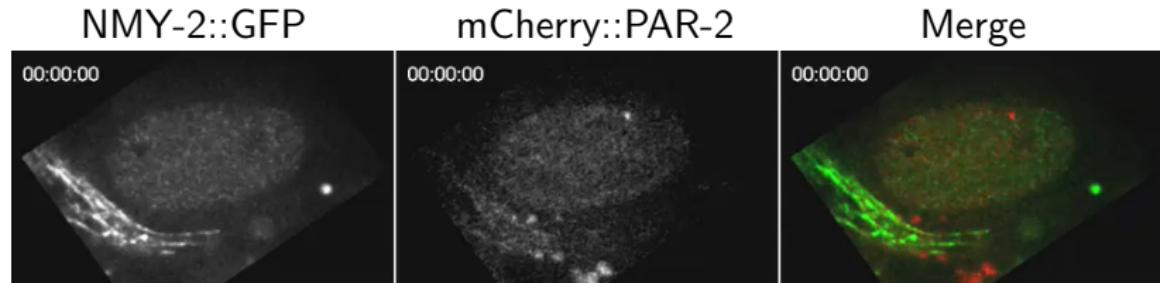
Disturb establishment by knocking down rho

- ▶ ECT-2 ts mutant → no cortical flows
- ▶ Both cases: local zone of PAR-2 enrichment remains

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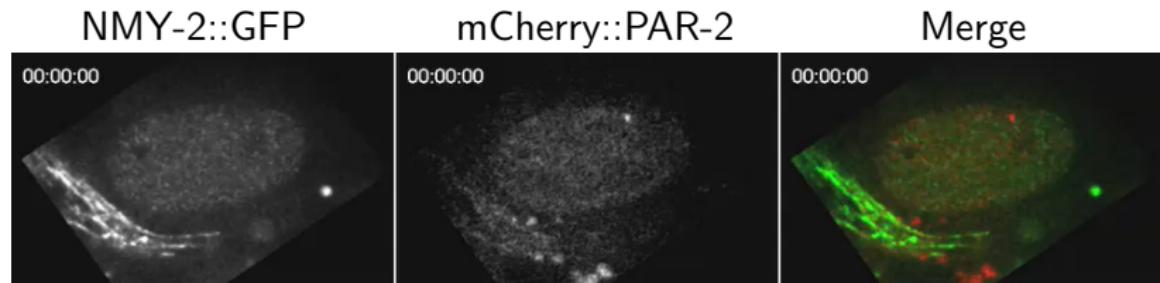
Results in exactly the same boundary position!

- ▶ Requires CDC-42, MRCK

Starting polarity from maintenance

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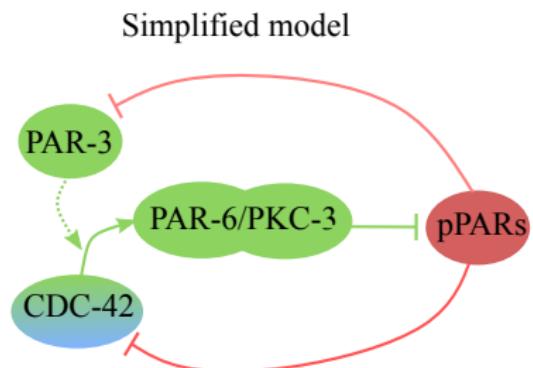
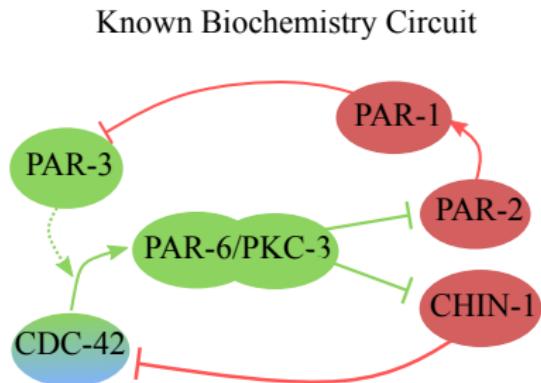
- ▶ Requires CDC-42, MRCK
- ▶ Experiments by Charlie show maintenance is an error correction mechanism

Questions to answer

Late maintenance: more myosin on anterior, steady A-directed flow

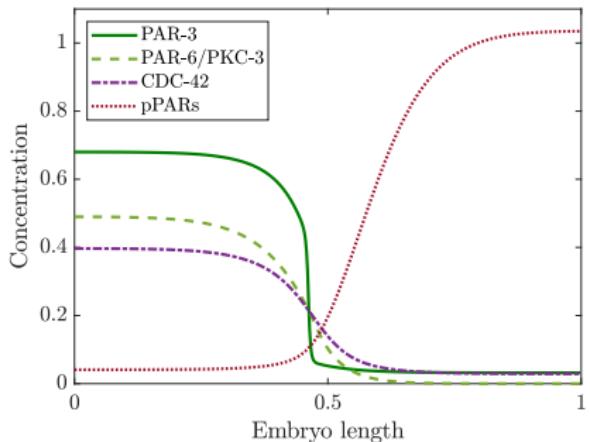
- ▶ Is this a steady state? ✓
- ▶ Is this an *attractive* steady state? Or does it depend on establishment? ✓
- ▶ Can we understand this with a simple model?
 - ▶ Account for aPAR/pPAR maintenance without flows
 - ▶ How to explain rescue?

Maintenance model

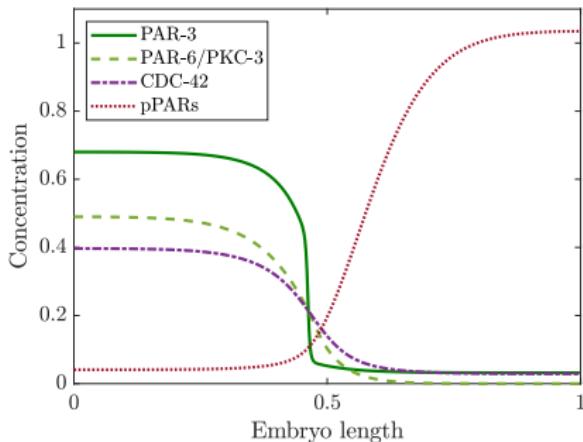


Maintenance model without flows

Starting from end establishment

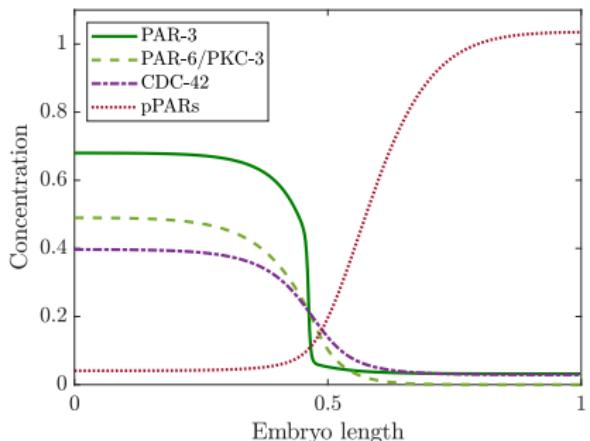


Starting from small asymmetry

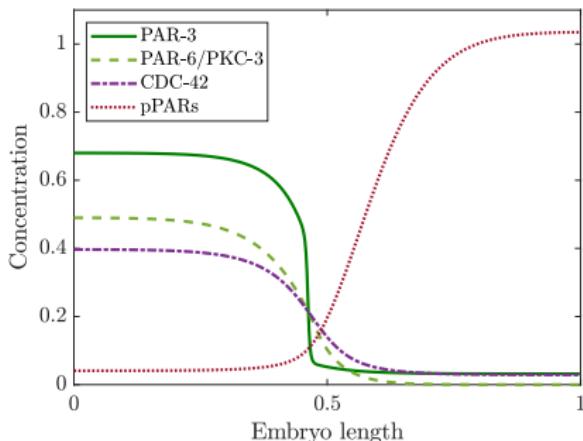


Maintenance model without flows

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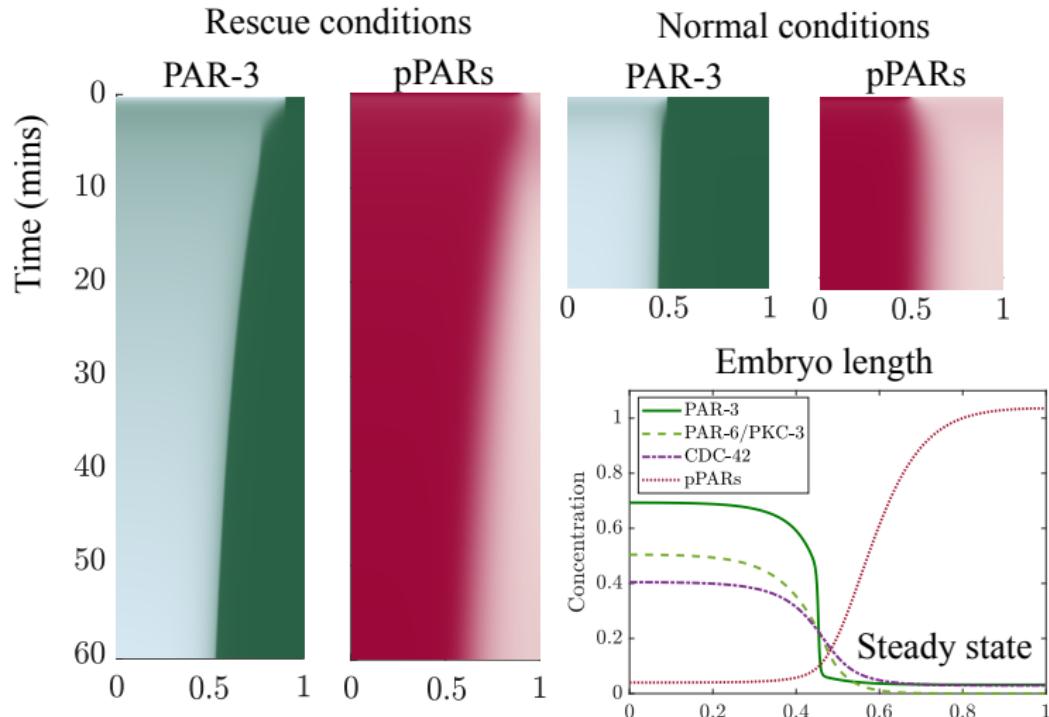
Starting from small asymmetry



Slow reaction-diffusion mechanism can “pin” boundary, but not in realistic time

- ▶ Consistent with experiment (MRCK = flows)

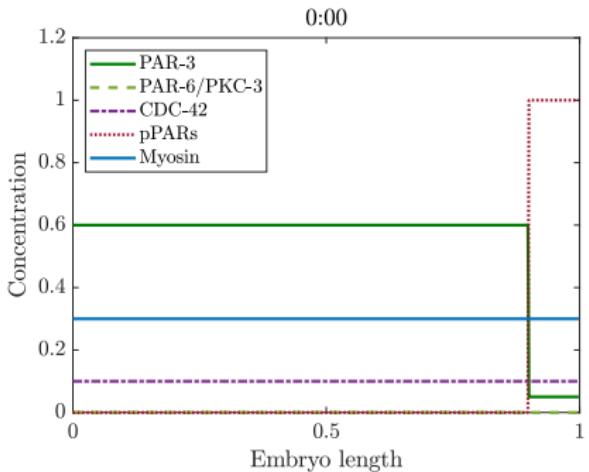
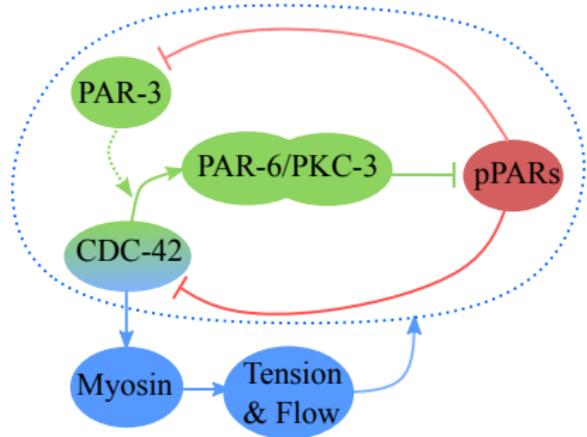
Maintenance model without flows: conclusions



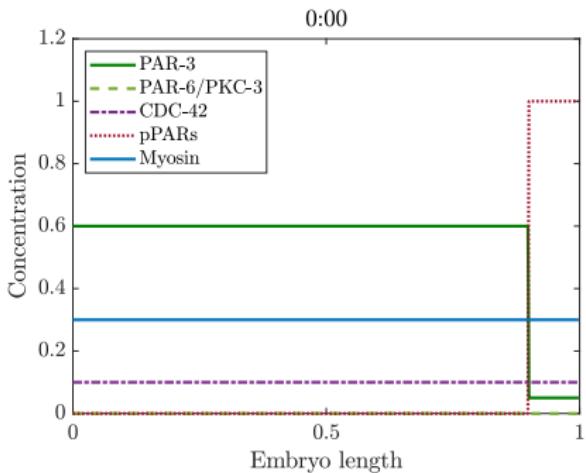
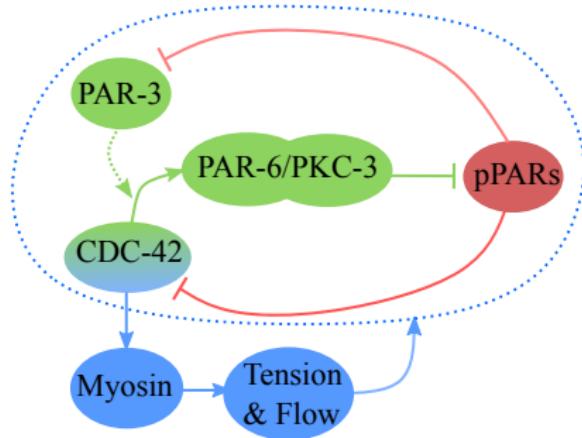
aPAR/pPAR circuit can maintain stable boundary, but cannot rescue it (in realistic time)

- ▶ Consistent with experiment (MRCK = flows)

Adding flows to maintenance model



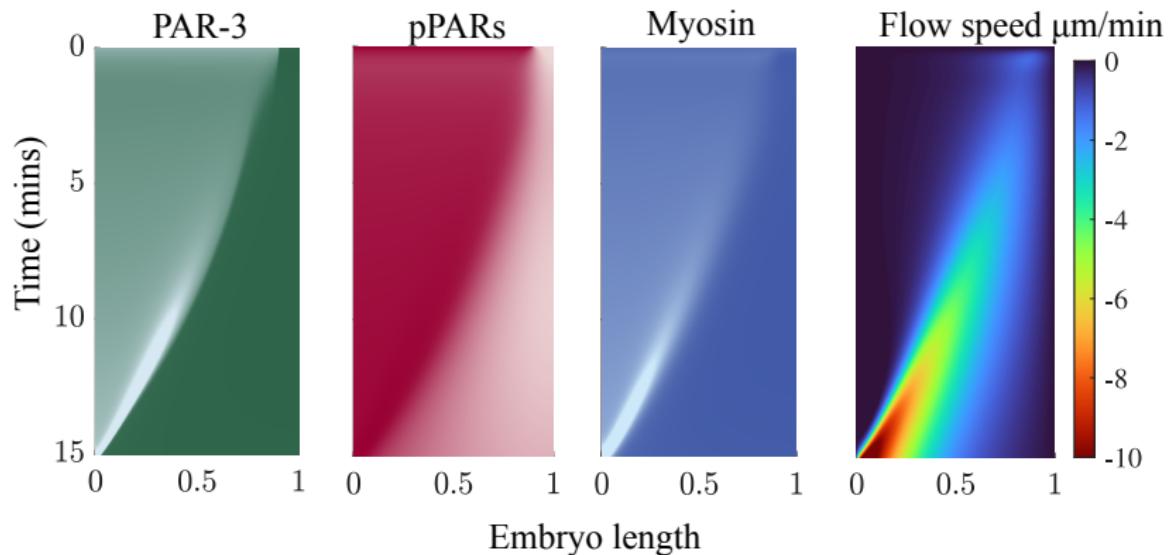
Adding flows to maintenance model



No way to stop the build up of myosin!

- ▶ Need some inhibitor of contractility on anterior

Adding flows to maintenance model: kymographs



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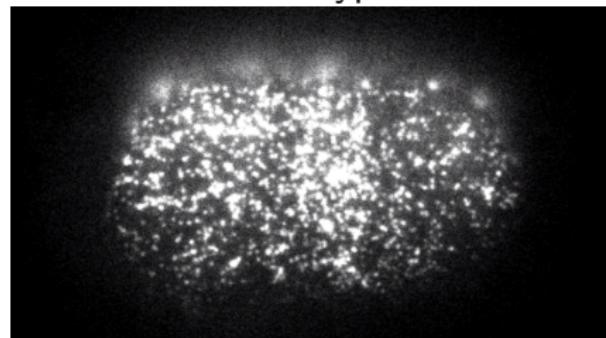
- ▶ Is this a steady state? ✓
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- ▶ Can we understand this with a simple model? X
- ▶ **Can experiments identify the missing piece?**
- ▶ Does a re-informed model make the correct predictions?

Depleting branched actin gives hypercontractile state

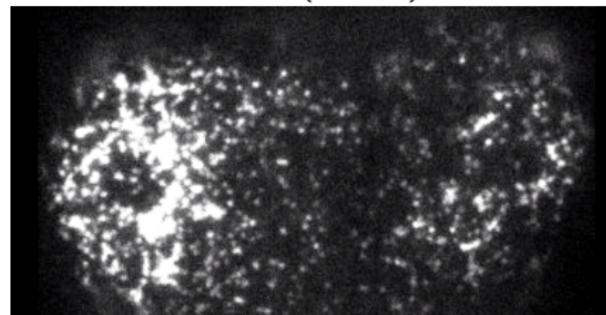
Treat with nocodazole to remove MT forces pulling on cap

- ▶ Hypothesis: branched actin halts myosin constriction
- ▶ Update movies

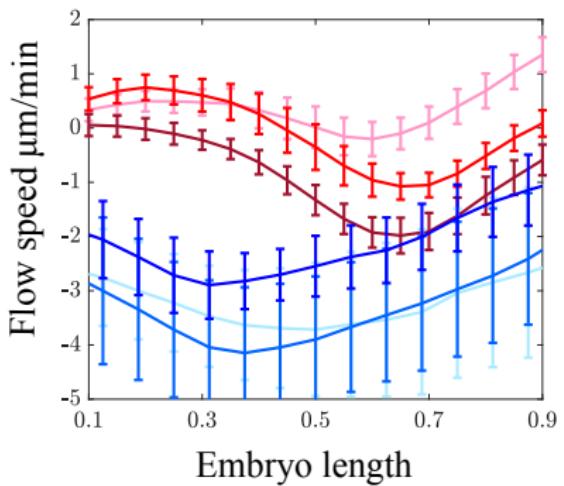
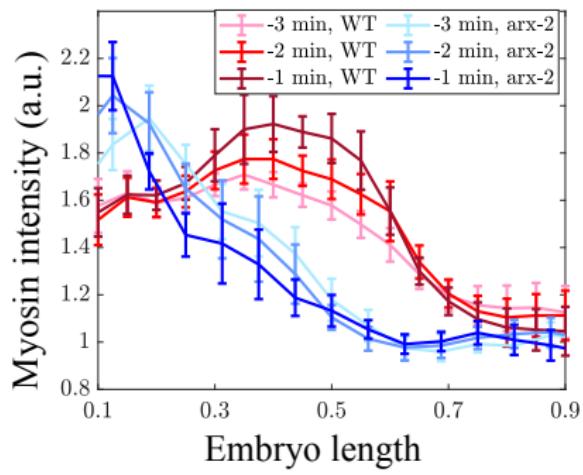
Wild type



arx-2 (RNAi)

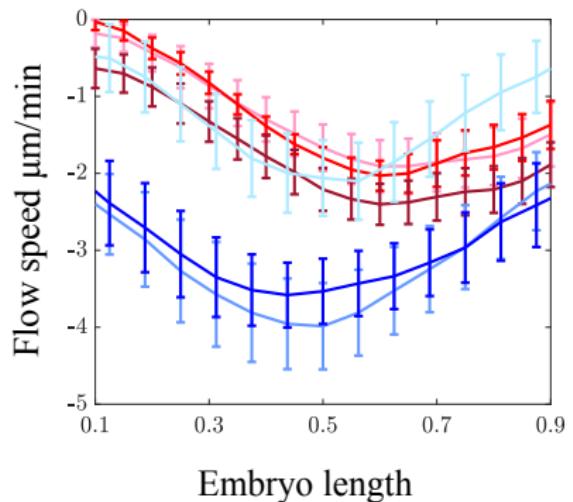
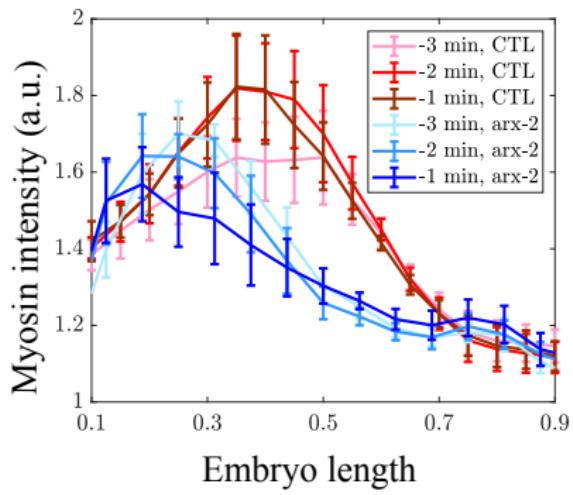


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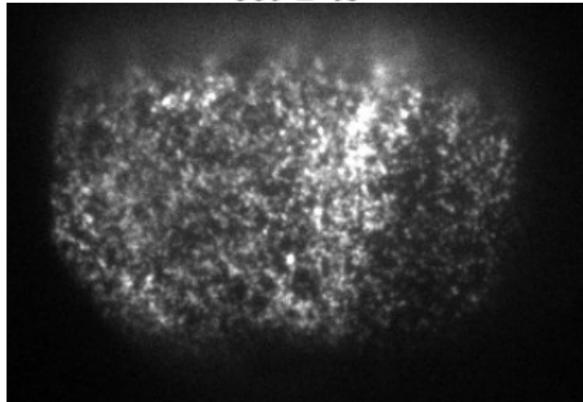
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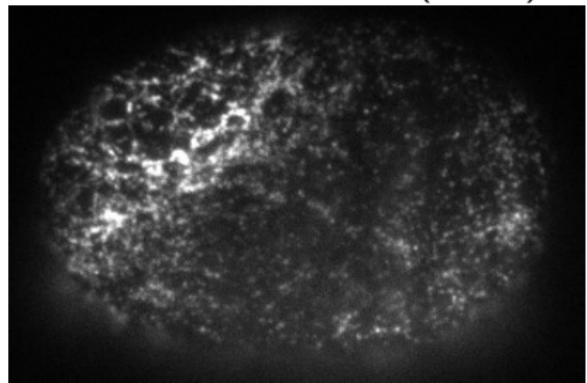


Does rescue work without branched actin?

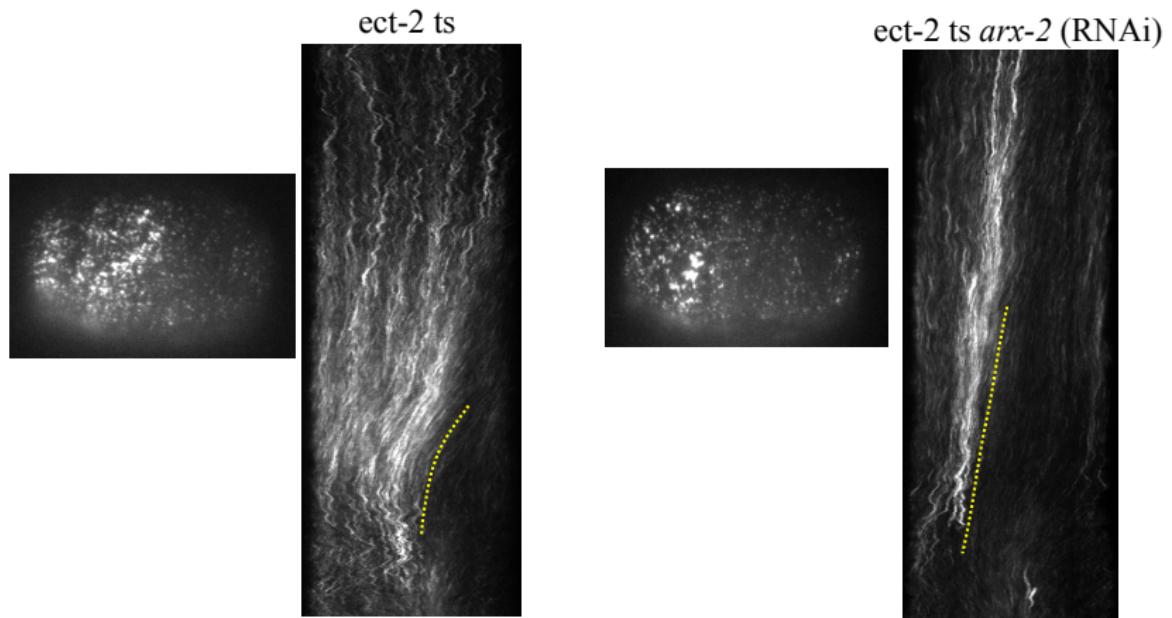
ect-2 ts



ect-2 ts + arx-2 (RNAi)



Does rescue work without branched actin?



Rescue dynamics: a closer look

Conclusions and future work

Maintenance phase “rescue” \approx same pathway as establishment

- ▶ PAR-3 intrinsically bistable
- ▶ PAR-2 “invades” based on excess cytoplasmic
- ▶ Myosin inhibition by PAR-2 \rightarrow further expansion
- ▶ Boundary pinned when run low on PAR-2

Flow and myosin profiles out of whack in WT vs. model

- ▶ Better match: *arx-2* (RNAi) embryos
- ▶ Branched actin inhibits contractility
- ▶ Incorporate into model; match WT?