

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

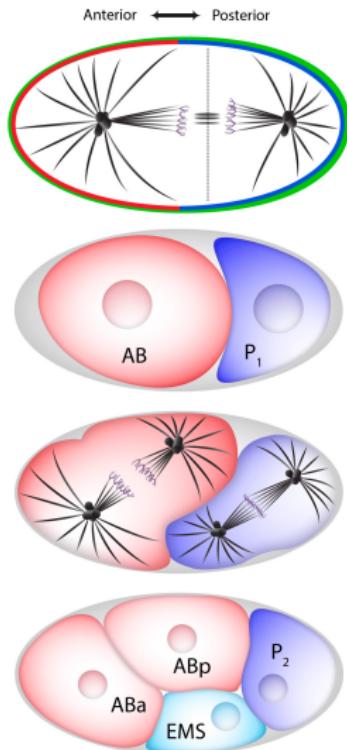
Ondrej Maxian, Cassandra Azeredo-Tseng, Ed Munro & Others

Munro Lab Group Meeting
April 15, 2024

Cell polarization

Spatial differences in protein concentration

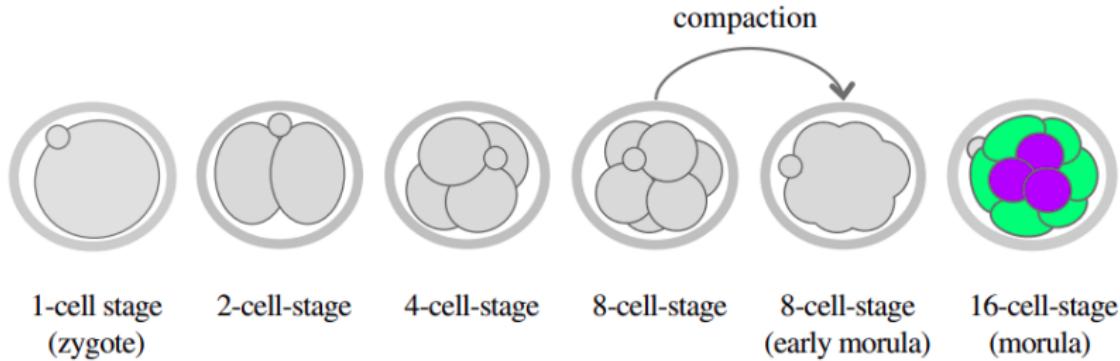
- ▶ Encode cell fate decisions
- ▶ Vital for proper development



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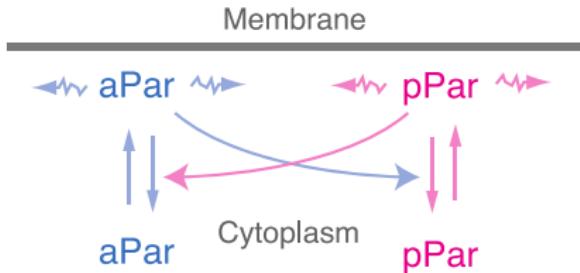
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One-cell *C. elegans* model system

Ingredients

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



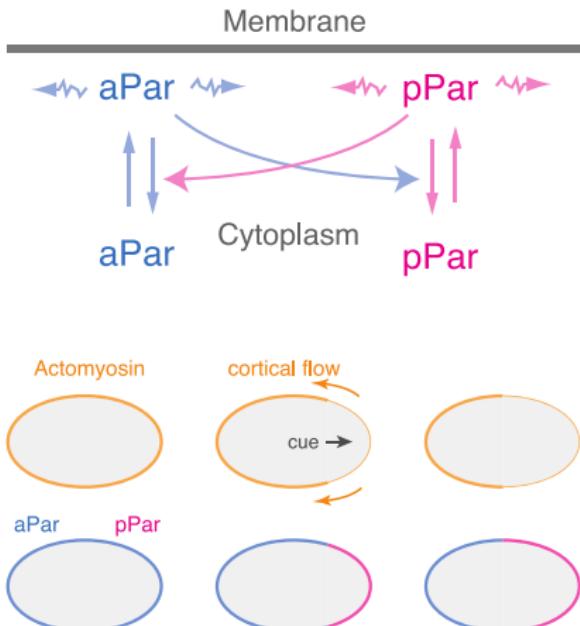
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Wild type sequence

- ▶ Centrosomes → pPARs loaded
- ▶ Sperm cue → Myosin inhibition
- ▶ Expansion of boundary to stable point (“establishment”)



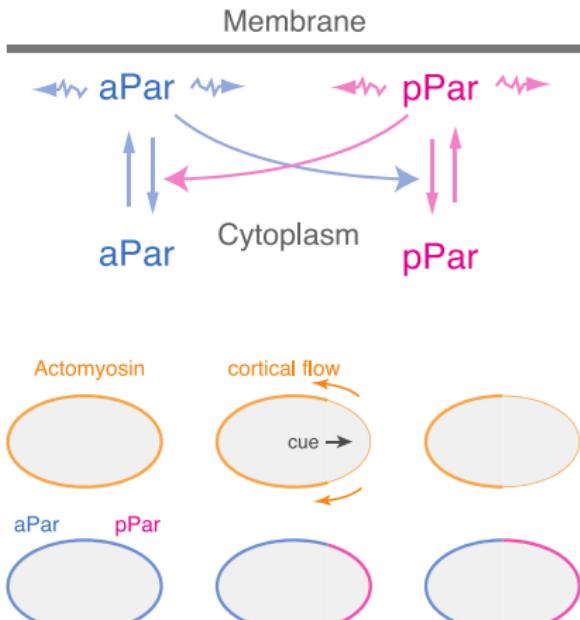
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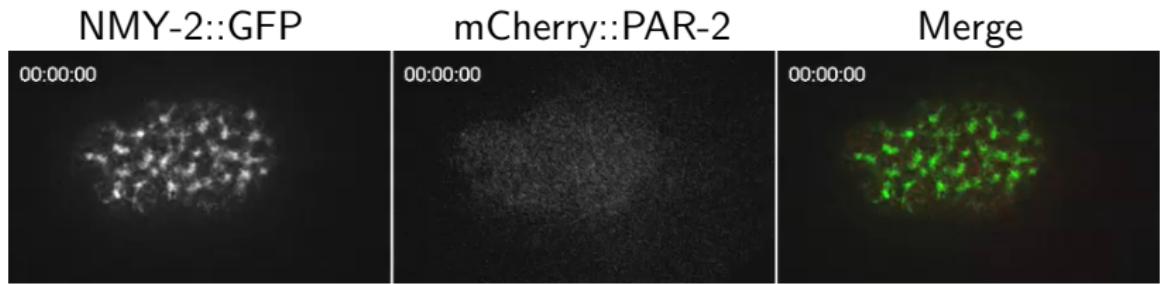
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Wild type sequence

- ▶ Centrosomes → pPARs loaded
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- ▶ Expansion of boundary to stable point ("establishment")
- ▶ "Maintenance:" boundary stays
- ▶ Focus: steady myosin/contractile asymmetry

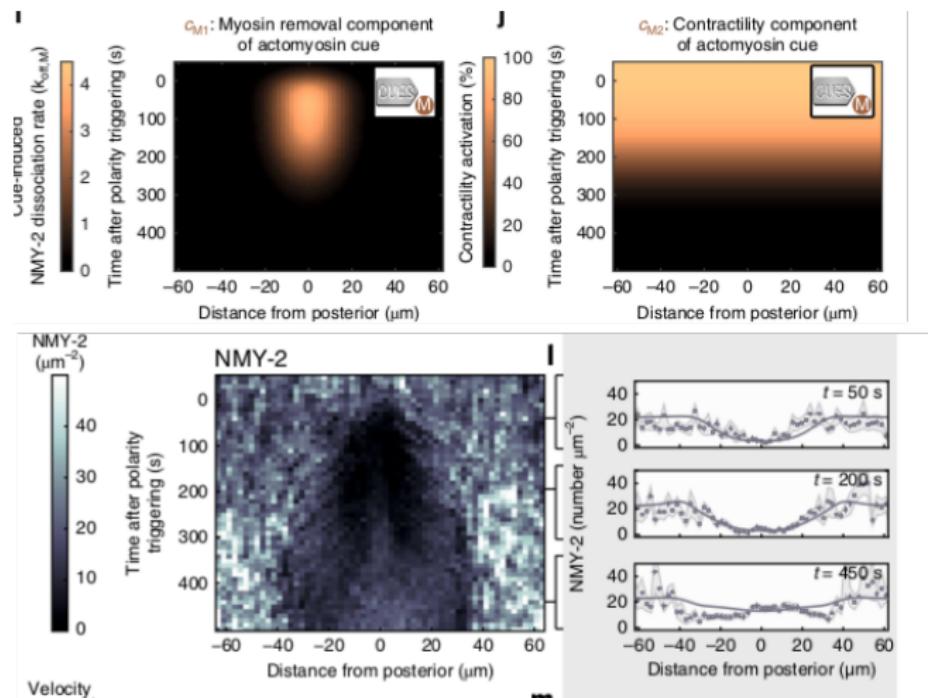


Movie: *C. elegans* wild type



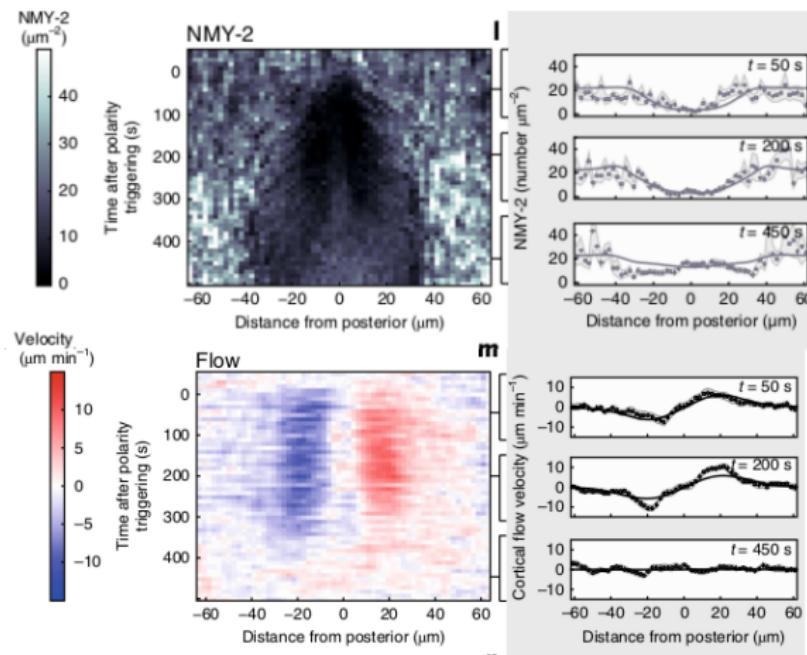
Standard version of the story: transient cues

Transient establishment phase cue generates myosin asymmetry, maintained at later times by PARs



Standard version of the story: steady flows?

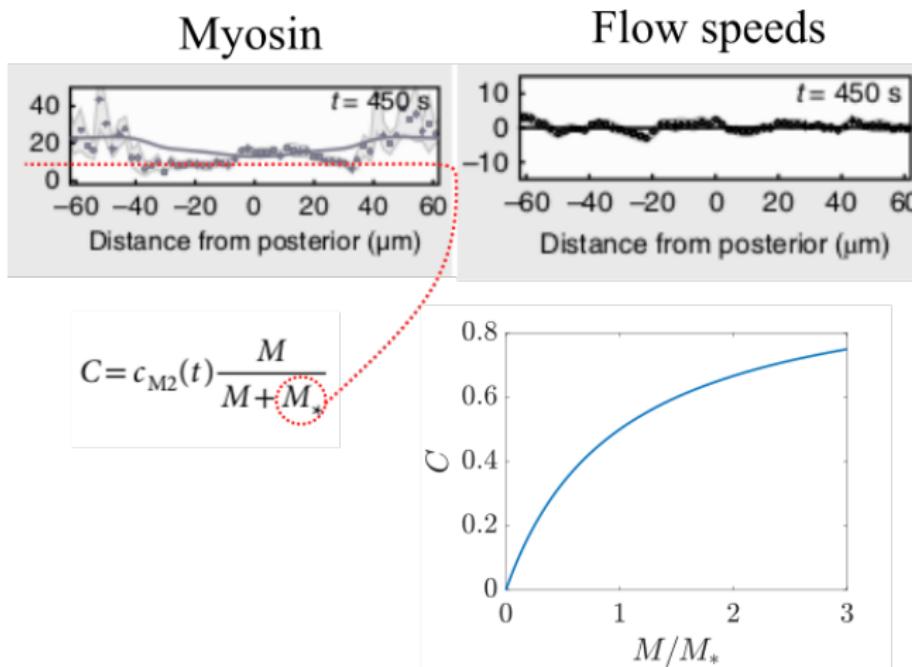
Q1: Despite steady myosin asymmetry, no flows in model?



Q2: Experiment shows shifting flow pattern: what to make of this?

Standard version of the story: why there are no flows

Q1: Contractility is saturated *everywhere!*



Q2: Shifting flow profile \rightarrow new phase

Equally disappointing?



ABC NEWS EXCLUSIVE

7:46 63°



"GOLDEN BACHELOR" COUPLE SPEAKS OUT

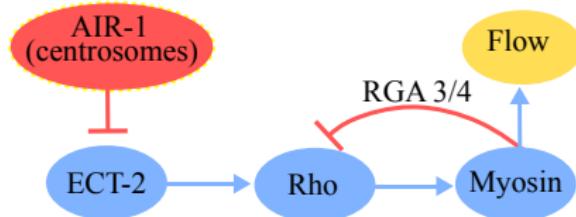
GERRY TURNER & THERESA NIST TO DIVORCE THREE MONTHS AFTER THEIR WEDDING

abc
NEWS

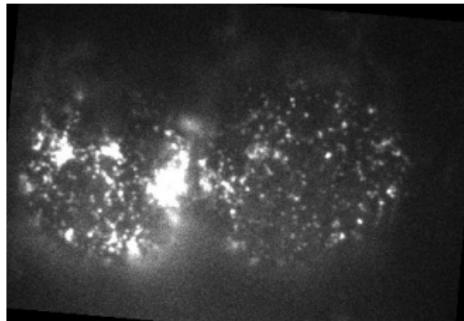


Distinction between establishment and maintenance

Establishment phase

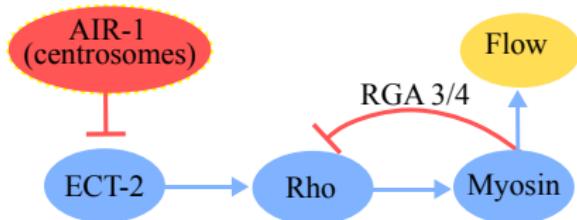


- ▶ Pulsatile contractility
(Michaux et al., 2018)
- ▶ Governed by rho

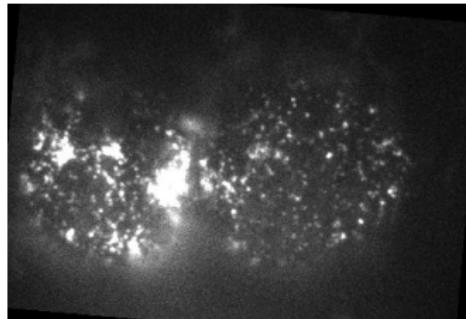


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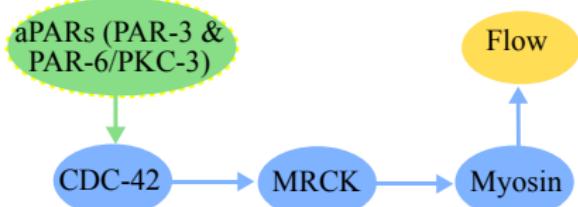
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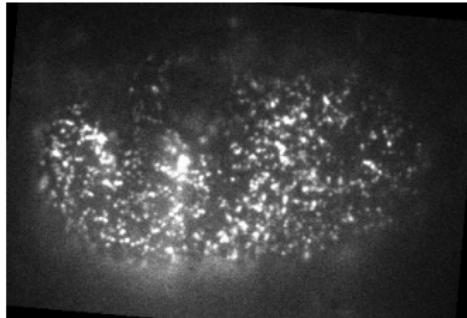
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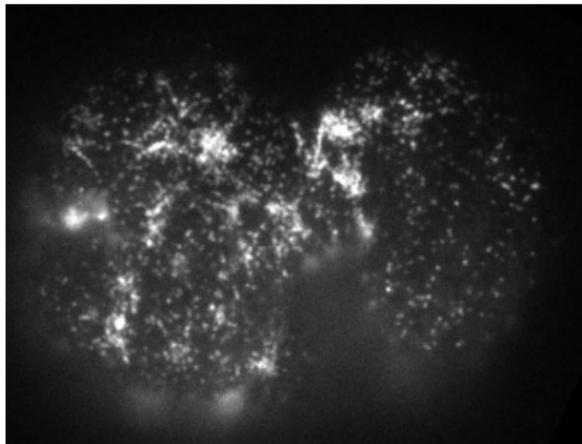
Maintenance phase



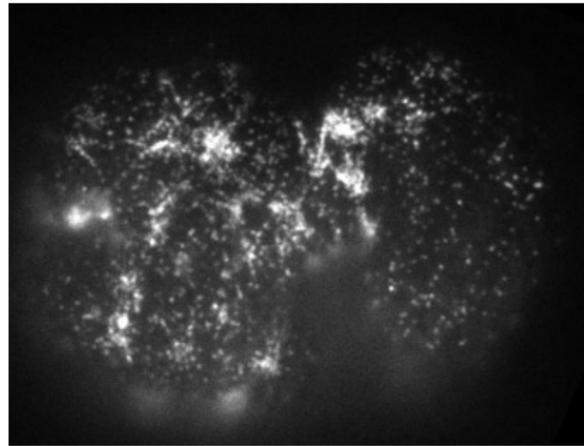
- ▶ Diffuse myosin clusters
- ▶ Governed by CDC-42
(through MRCK)



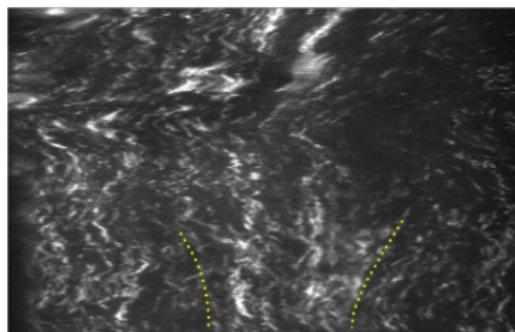
Maintenance phase: a closer look



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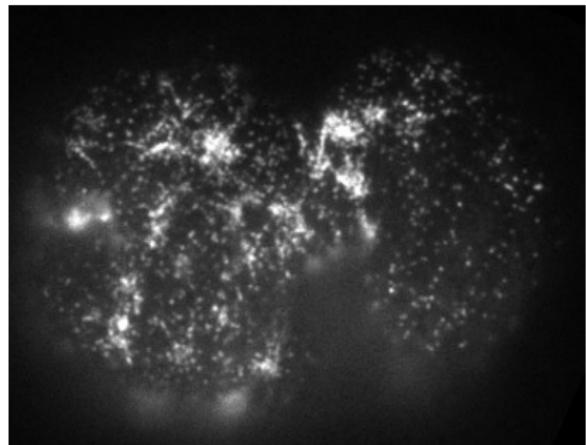


$t = 0$
mins

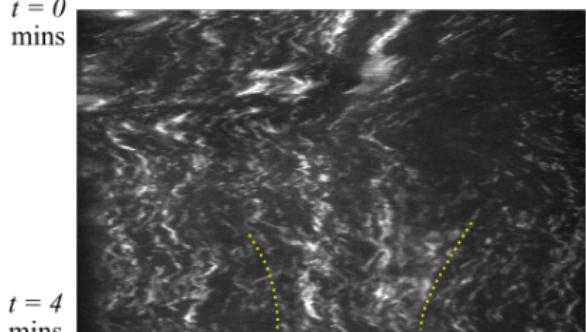


$t = 4$
mins

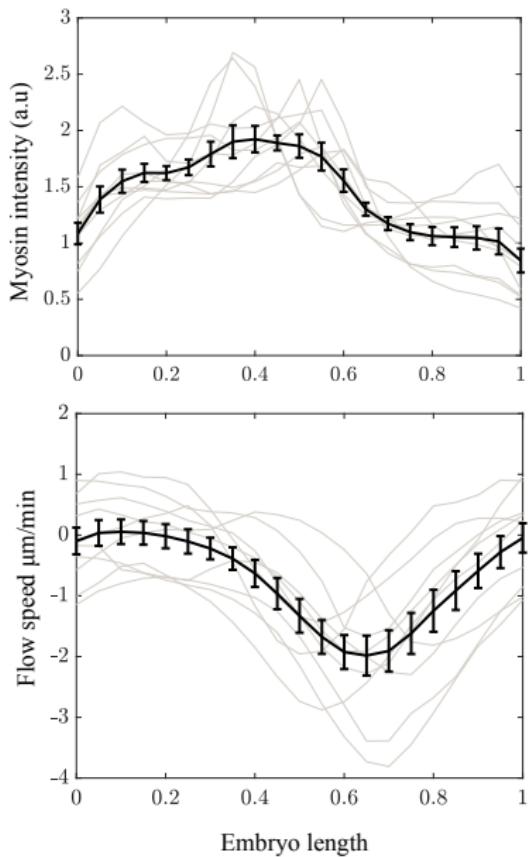
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Questions to answer

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

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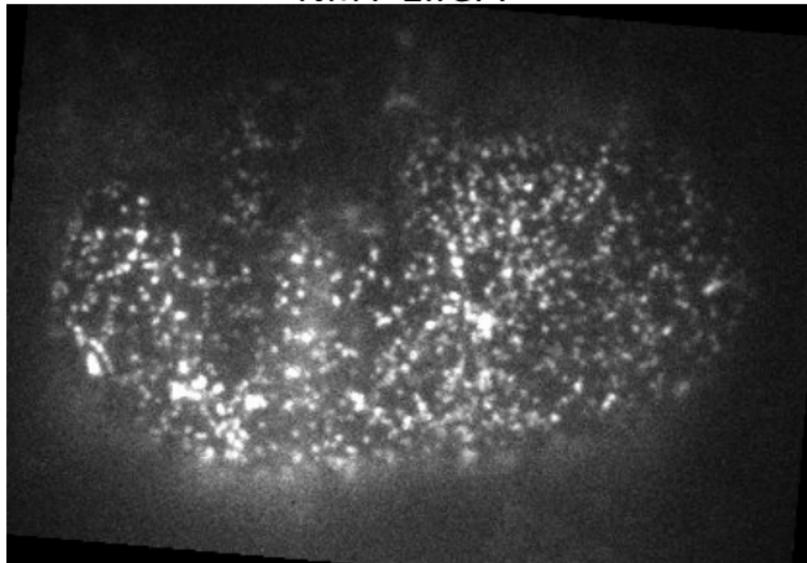
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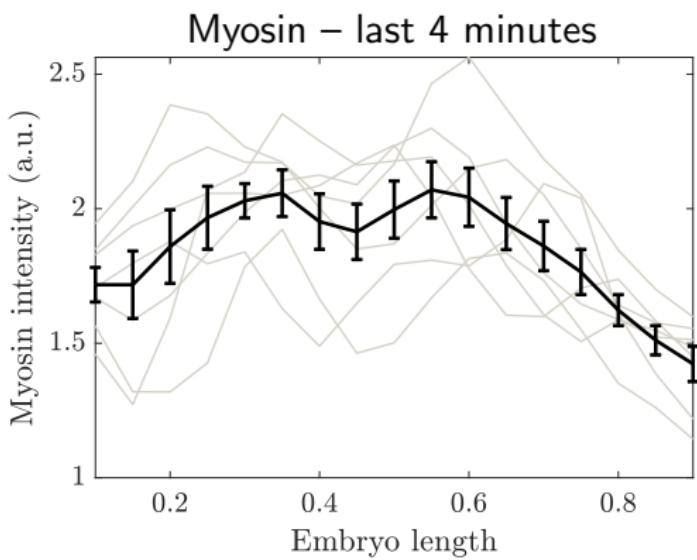
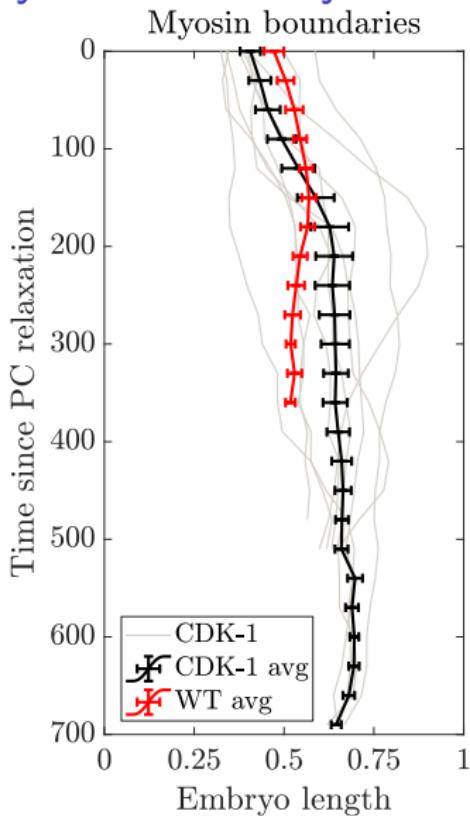
Late maintenance is (roughly) a steady state

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

NMY-2::GFP

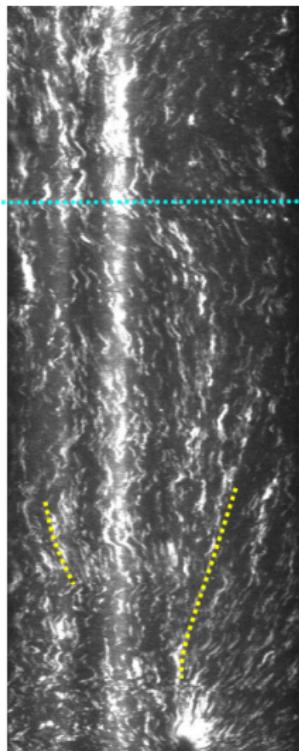


Myosin boundary stable

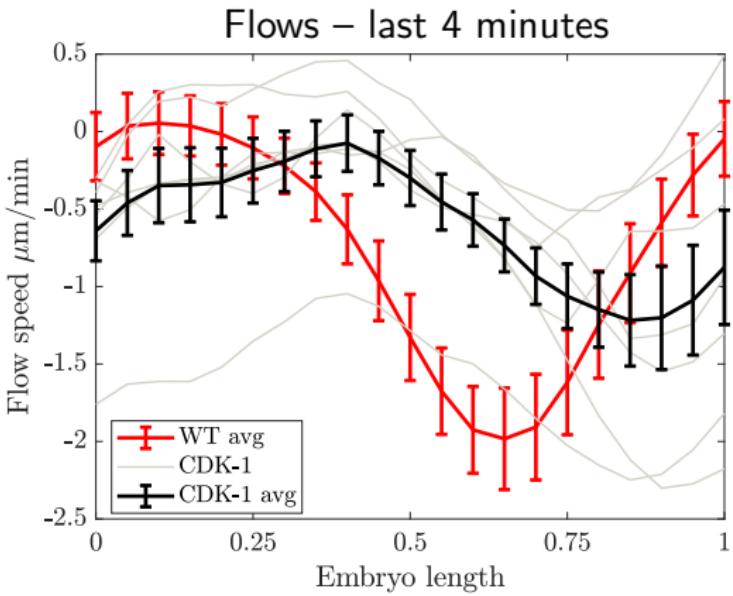


Slight shift towards posterior; but still have A/P asymmetry

Steady flow profile in CDK-1 embryos



Late est Maintenance (12 mins)



Still see contraction into medial domain (nonzero flows)

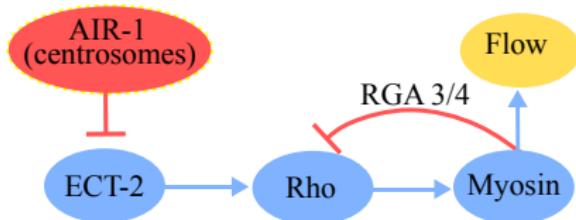
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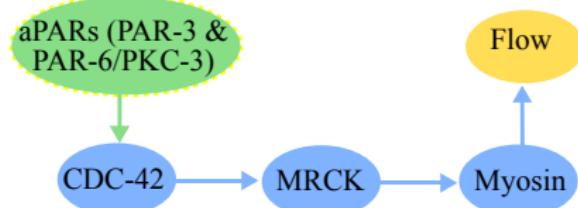
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Distinction between establishment and maintenance

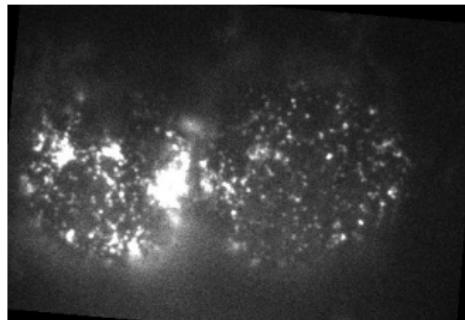
Establishment phase



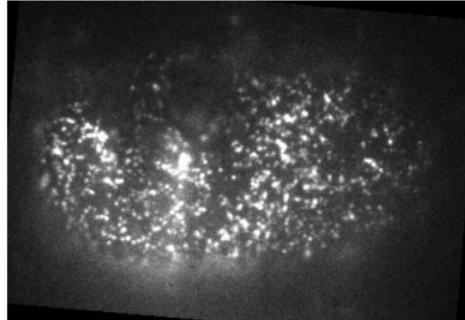
Maintenance phase



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Starting polarity from maintenance

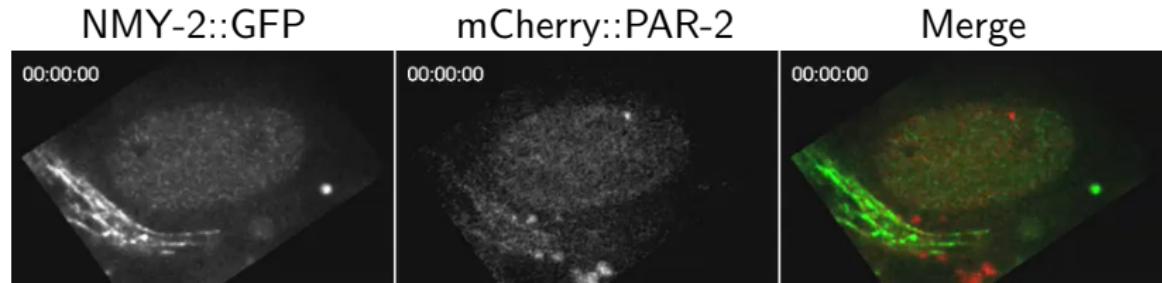
Disturb establishment by knocking down rho

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

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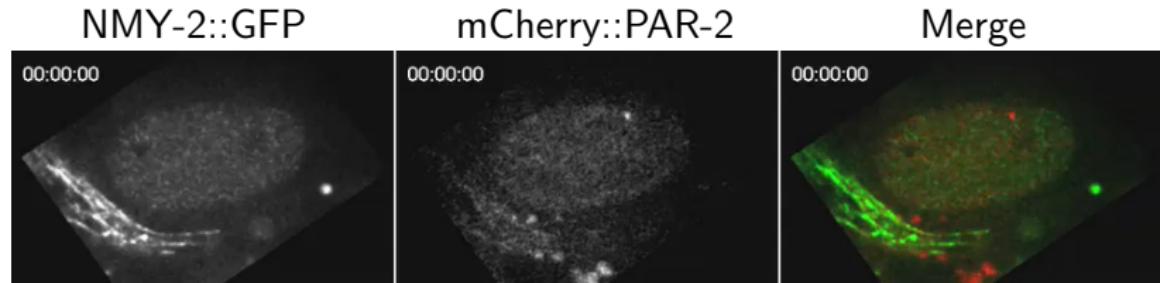
Results in exactly the same boundary position!

- ▶ Requires CDC-42, MRCK

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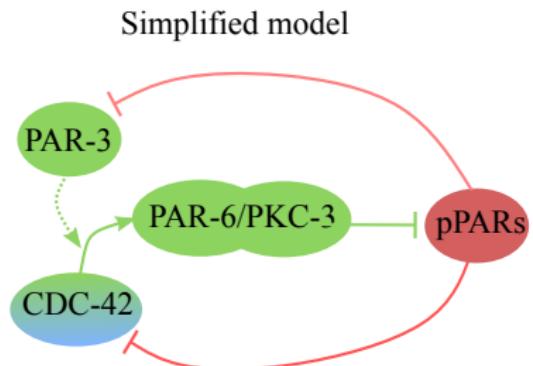
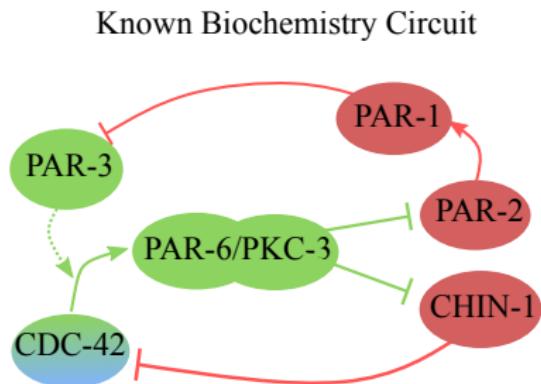
- ▶ Requires CDC-42, MRCK
- ▶ Experiments by Charlie show maintenance is an error correction mechanism

Questions to answer

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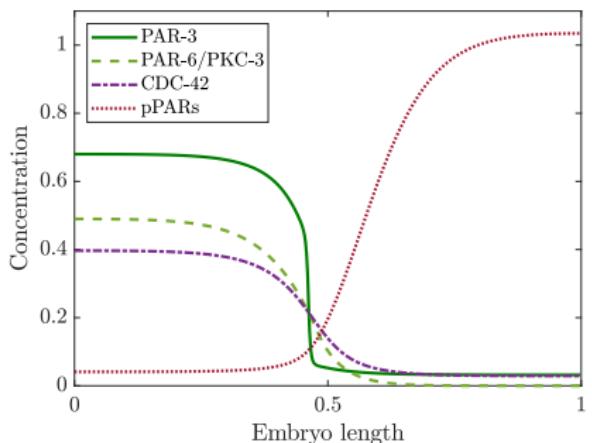
- ▶ Is this a steady state? ✓
- ▶ Is this an *attractive* steady state? Or does it depend on establishment? ✓
- ▶ Can we understand this with a simple model?
 - ▶ Account for aPAR/pPAR maintenance without flows
 - ▶ How to explain rescue?

Maintenance model

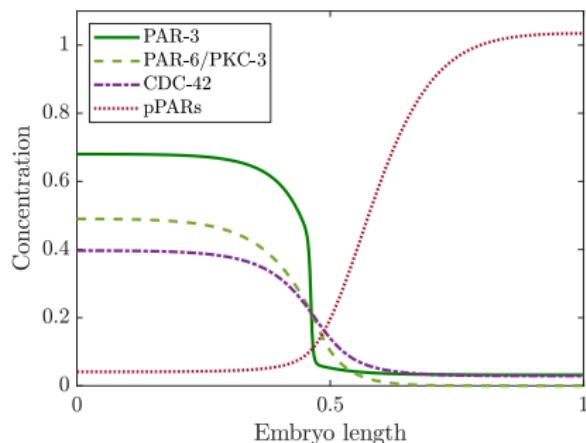


Maintenance model without flows

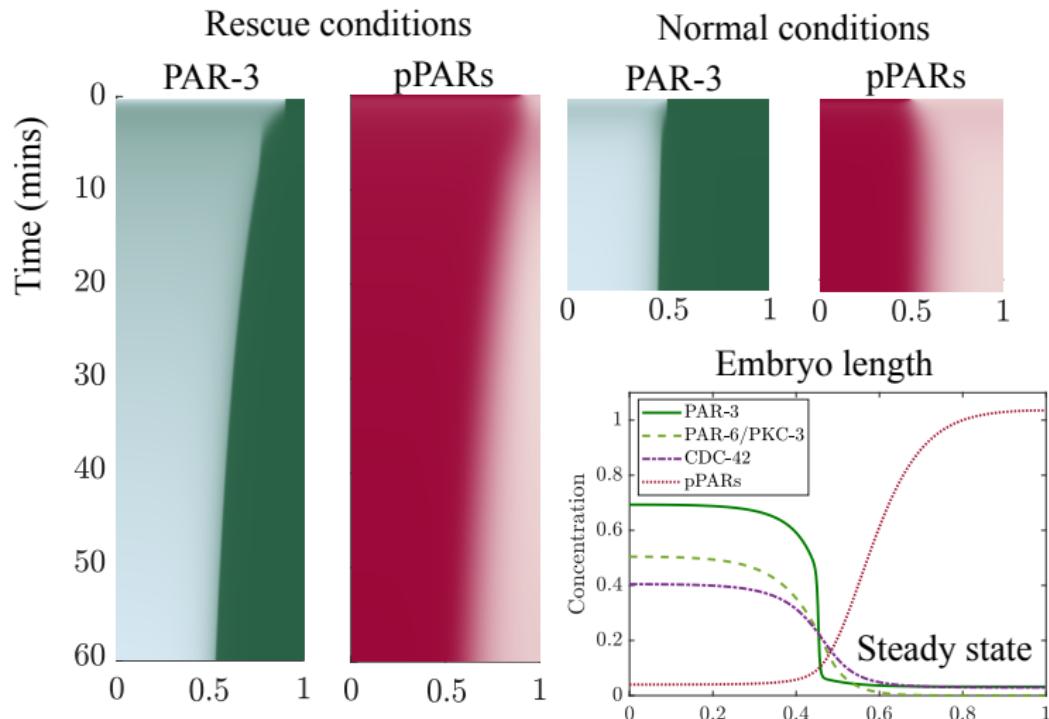
Starting from end establishment



Starting from small asymmetry



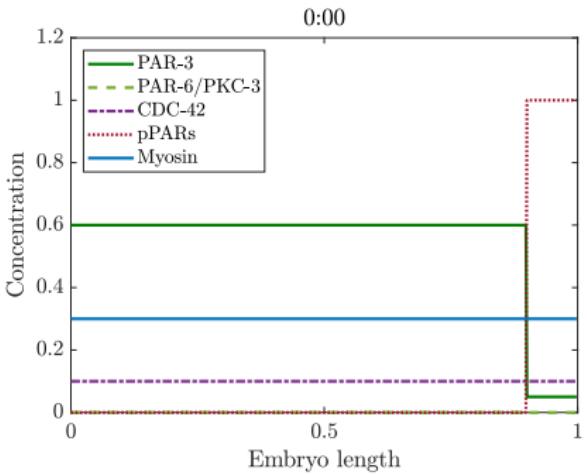
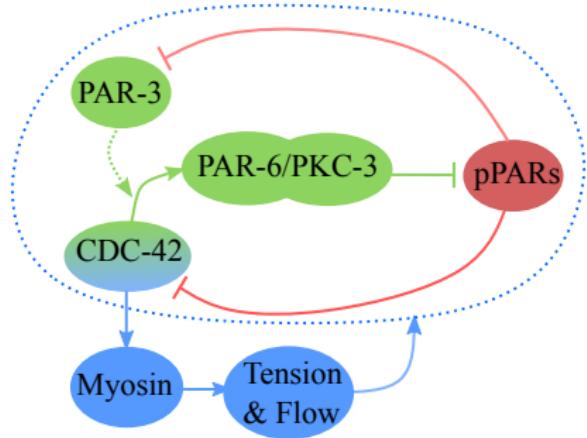
Maintenance model without flows: conclusions



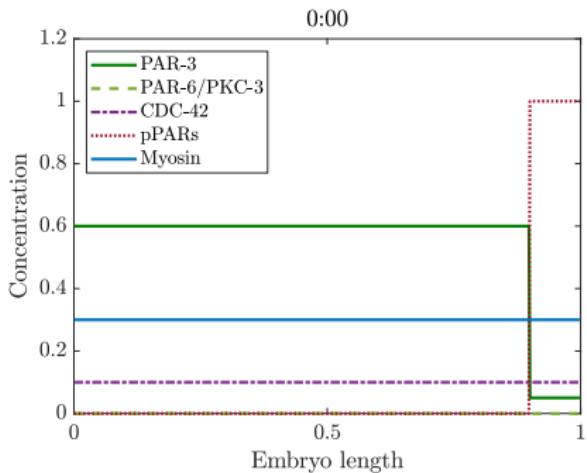
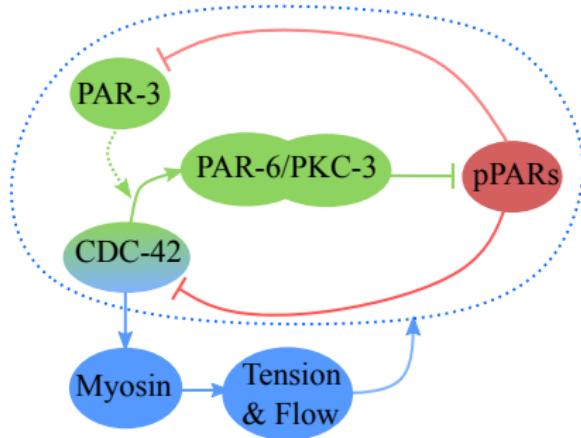
aPAR/pPAR circuit can maintain stable boundary, but cannot rescue it (in realistic time)

- ▶ Consistent with experiment (MRCK = flows)

Adding flows to maintenance model



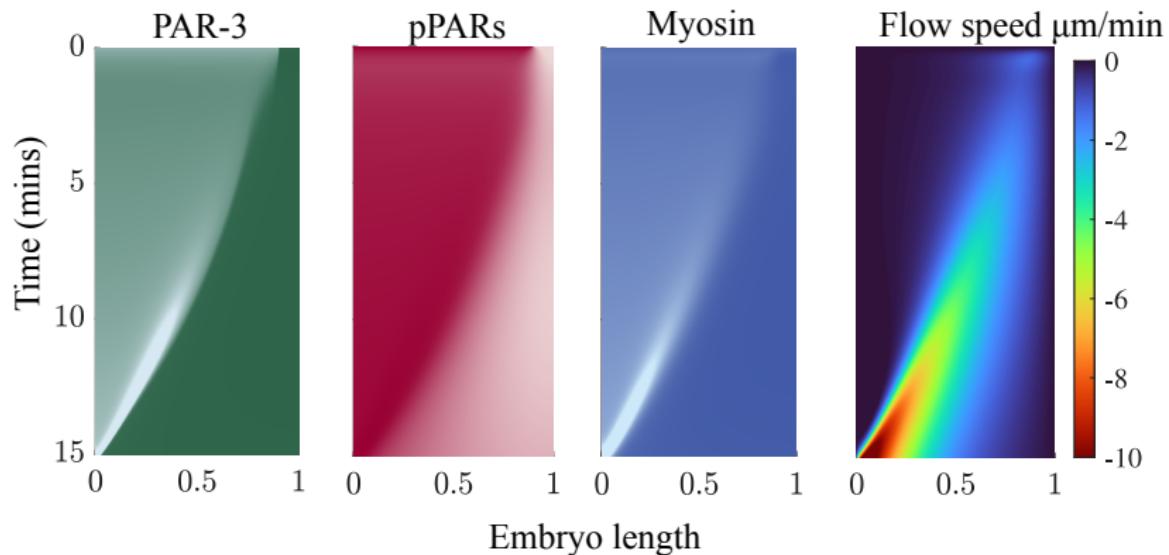
Adding flows to maintenance model



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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Questions to answer

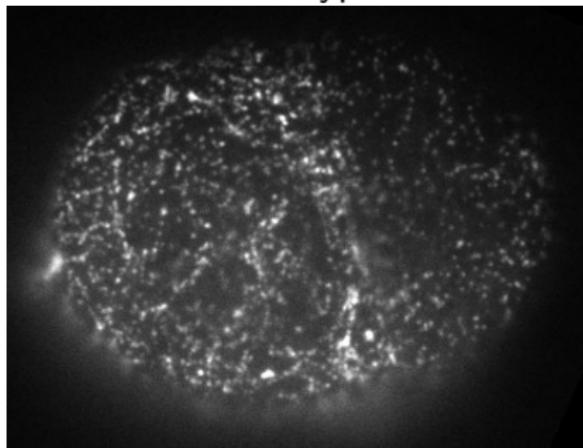
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- ▶ Can we understand this with a simple model? X
- ▶ **Can experiments identify the missing piece?**
- ▶ Does a re-informed model make the correct predictions?

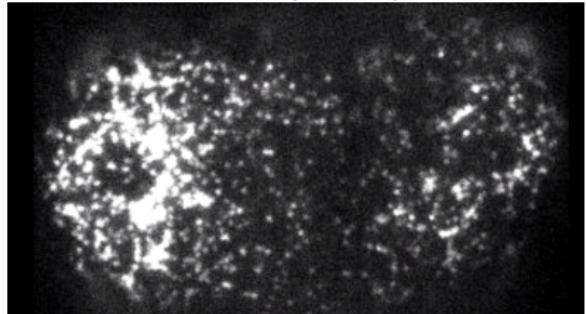
Depleting branched actin gives hypercontractile state

Hypothesis: branched actin halts myosin constriction

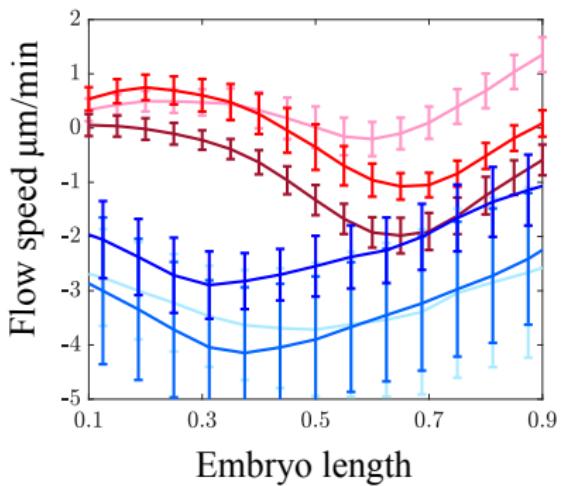
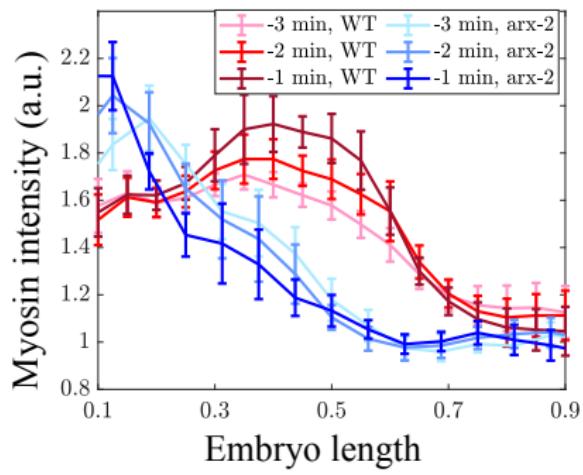
Wild type



arx-2 (RNAi)

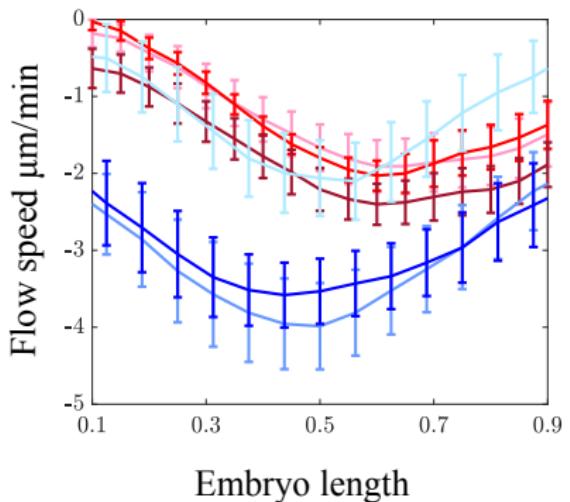
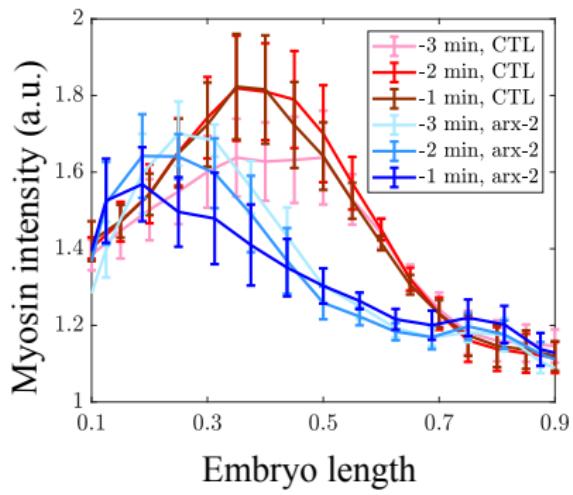


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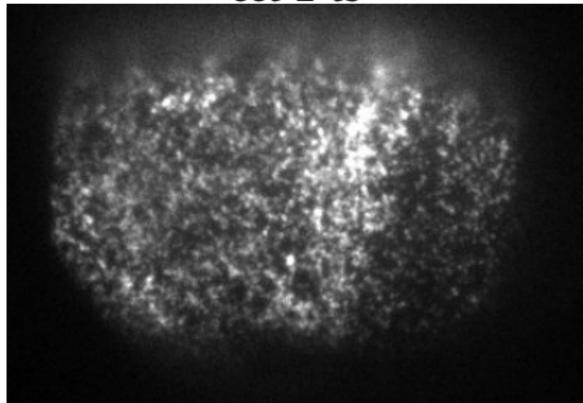
Depleting branched actin gives hypercontractile state

Treat with nocodazole to remove MT forces pulling on cap

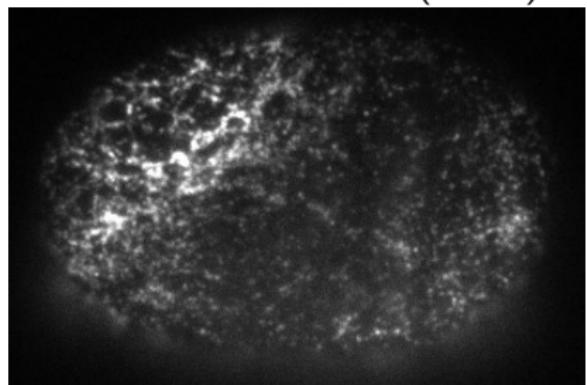


Does rescue work without branched actin?

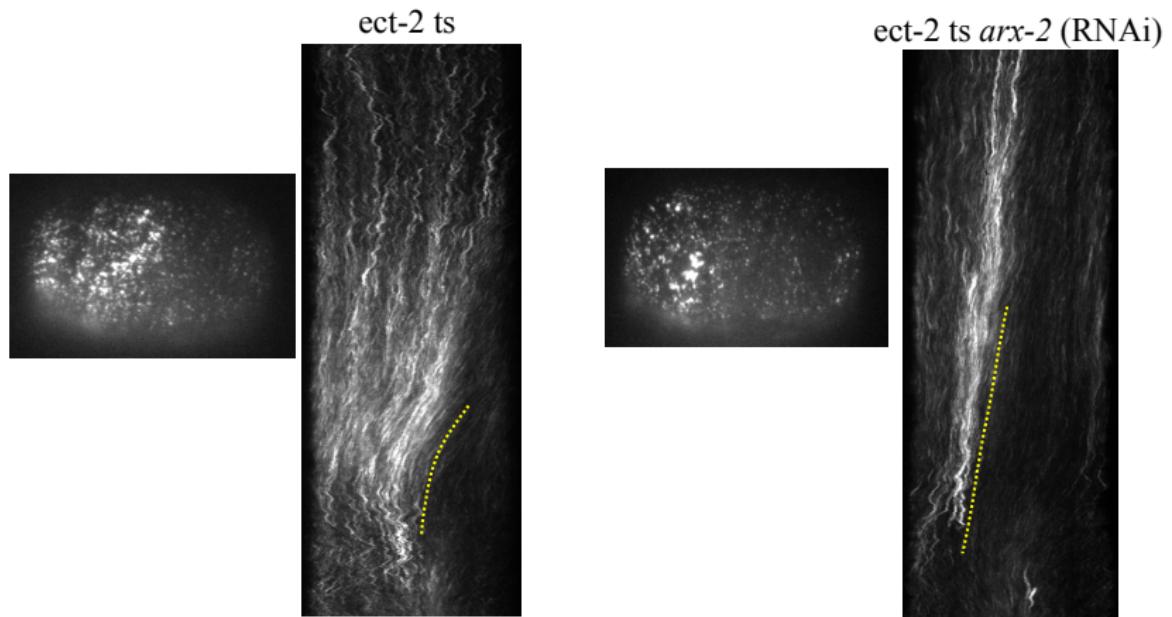
ect-2 ts



ect-2 ts + arx-2 (RNAi)



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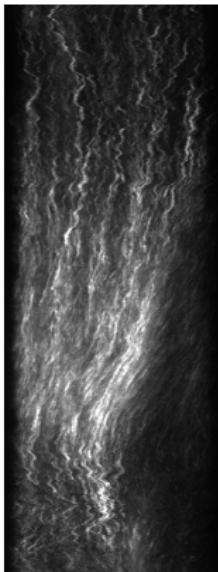
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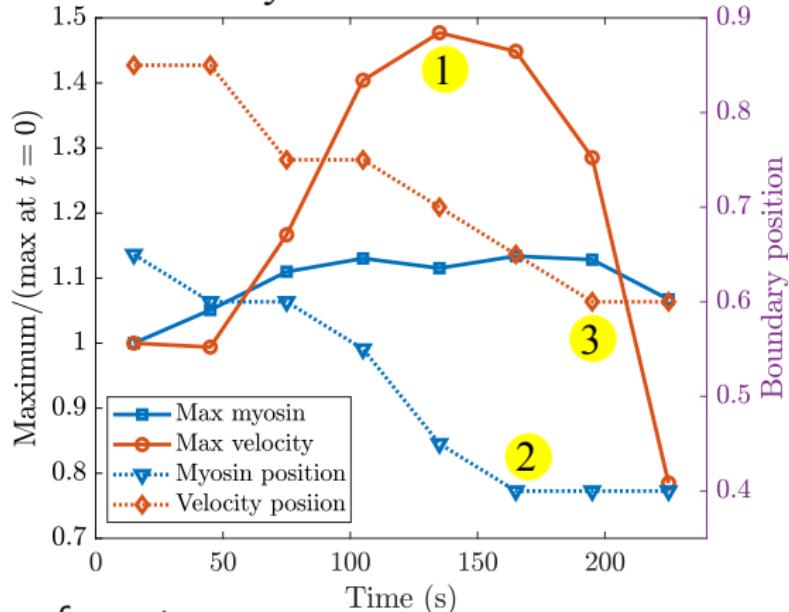
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Rescue dynamics: a closer look

ect-2 ts



Dynamics of rescue



Signature sequence of events

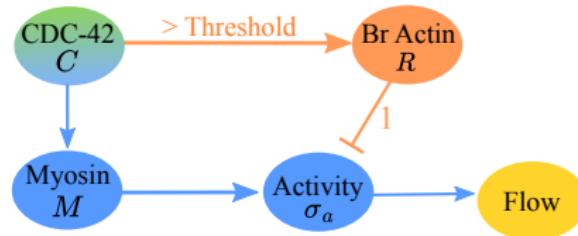
1. Sudden stalling and decrease of flow
2. Myosin boundary stops
3. Flow maximum fixed

No change in myosin peak!

Modeling the effect of branched actin

Experiments suggest sudden relief of anterior tension

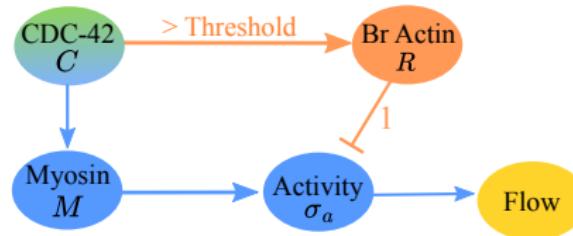
- Model as CDC-42 threshold where branched actin is activated



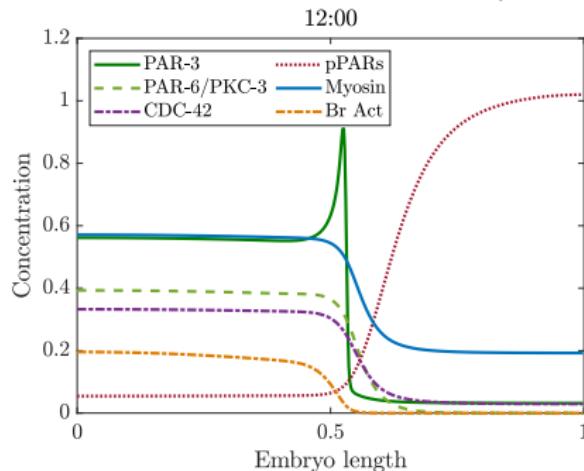
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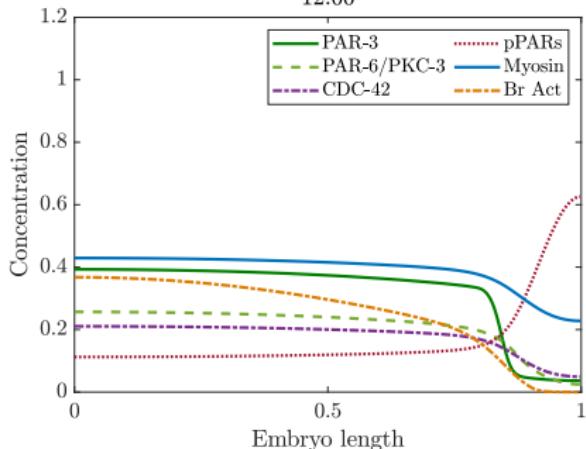


- Idea: threshold has to be between the A/P CDC-42 levels

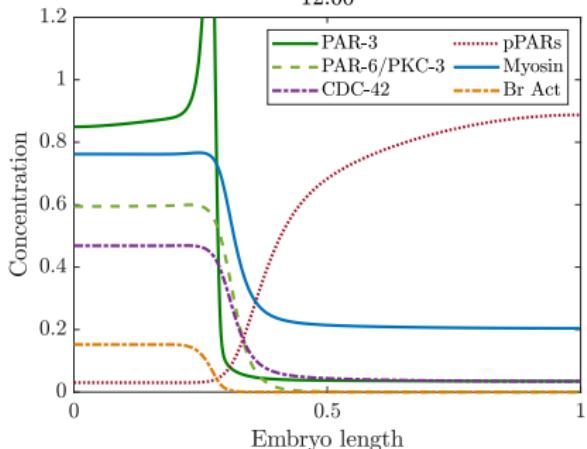


Changing the threshold alters state

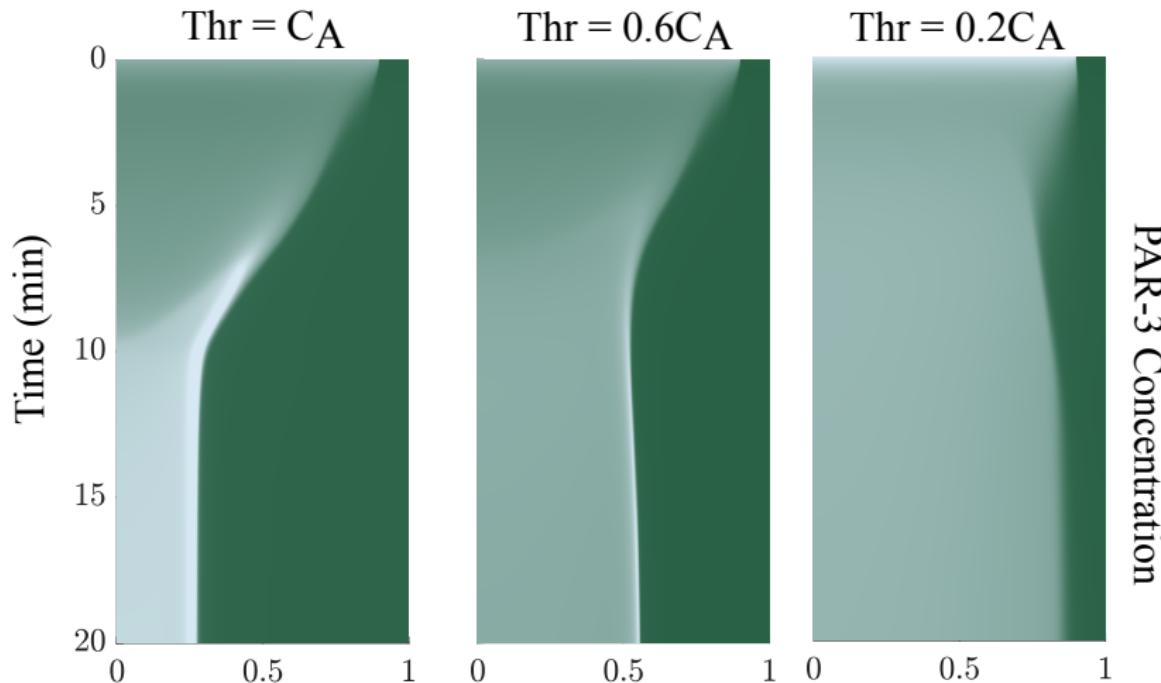
Thres = $0.2c_A$ (noncontractile)



Thres = c_A (hypercontractile)

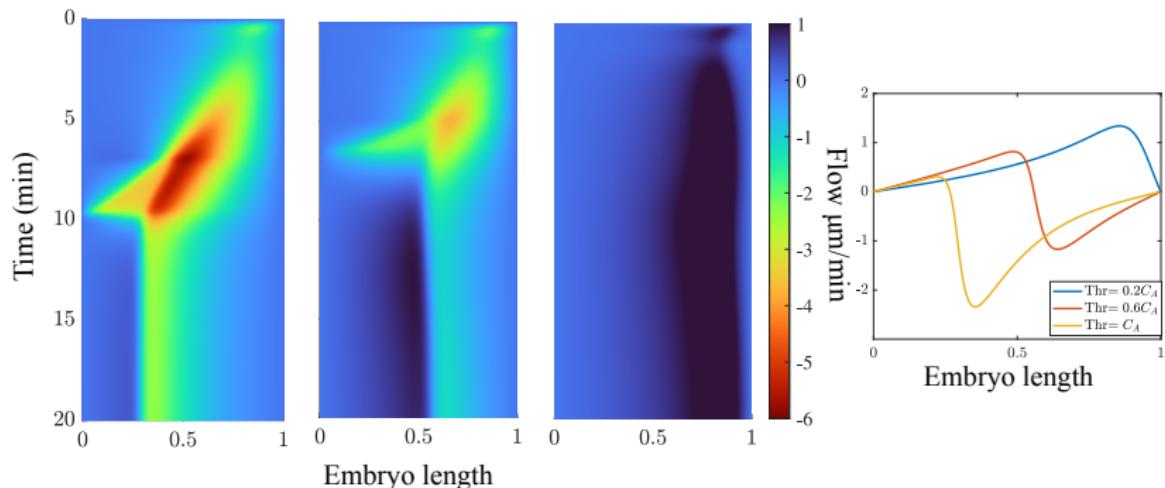


Summary kymographs: polarity markers



Size of anterior domain regulated by CDC-42 threshold

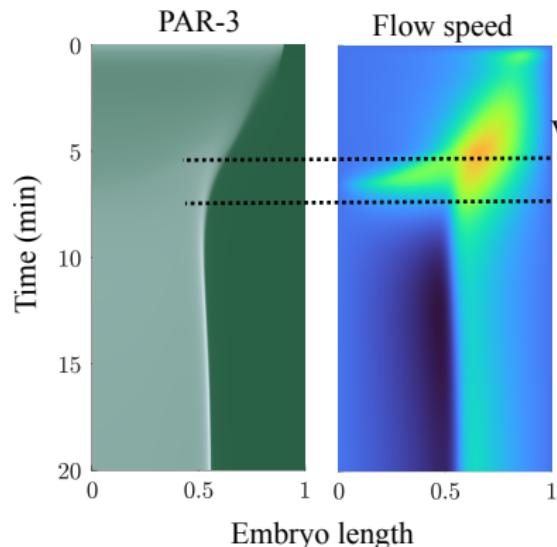
Summary kymographs: flows



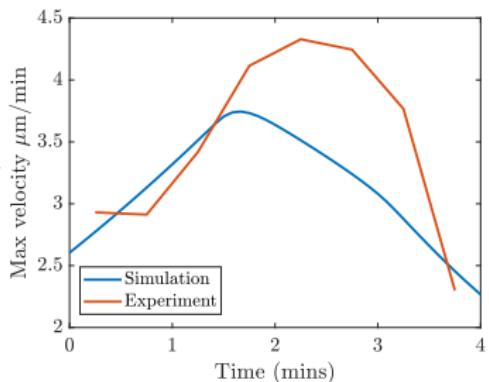
Steady A/P asymmetry in flow; dependent on size of threshold

- ▶ Advection flux balanced by diffusion at steady state

Mid-threshold: comparing with experiments



Velocity slows
Boundary pinned



Not reproducing sharp dependence, but qualitatively right dynamics

Conclusions

Maintenance phase encodes dynamically stable state

- ▶ Persistent A-directed flow
- ▶ Asymmetric myosin
- ▶ Can't be reproduced in simple models (runaway train)

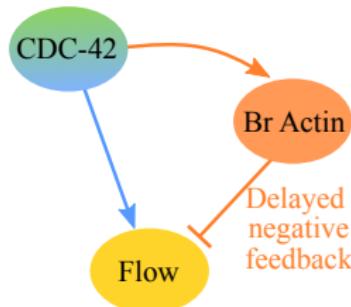
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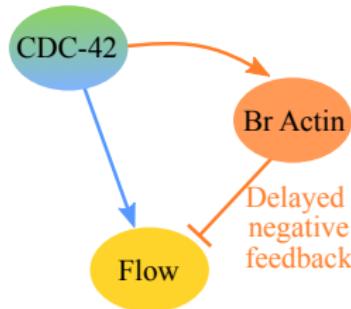
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- ▶ Flows transport boundary, branched actin slows down flows, reaction/diffusion sets final position