Maintenance phase biochemistry and contractility combine to encode a dynamically stable polarity state in the *C elegans* zygote

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Munro Lab Group Meeting
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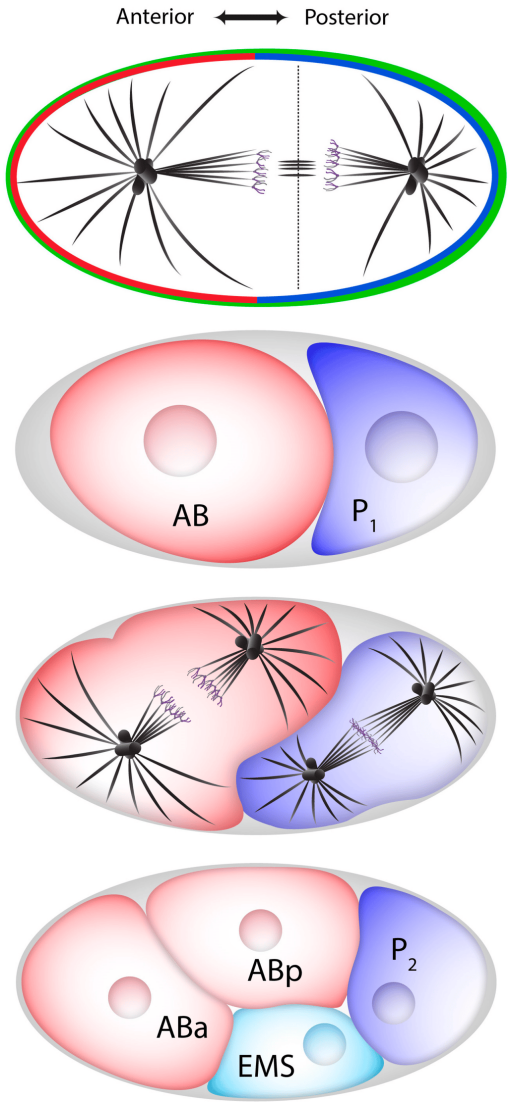
Cell polarization

Spatial differences in protein concentration

- Encode cell fate decisions
- Vital for proper development



Dewey et. al, *J. Dev. Biol.*, 2015.

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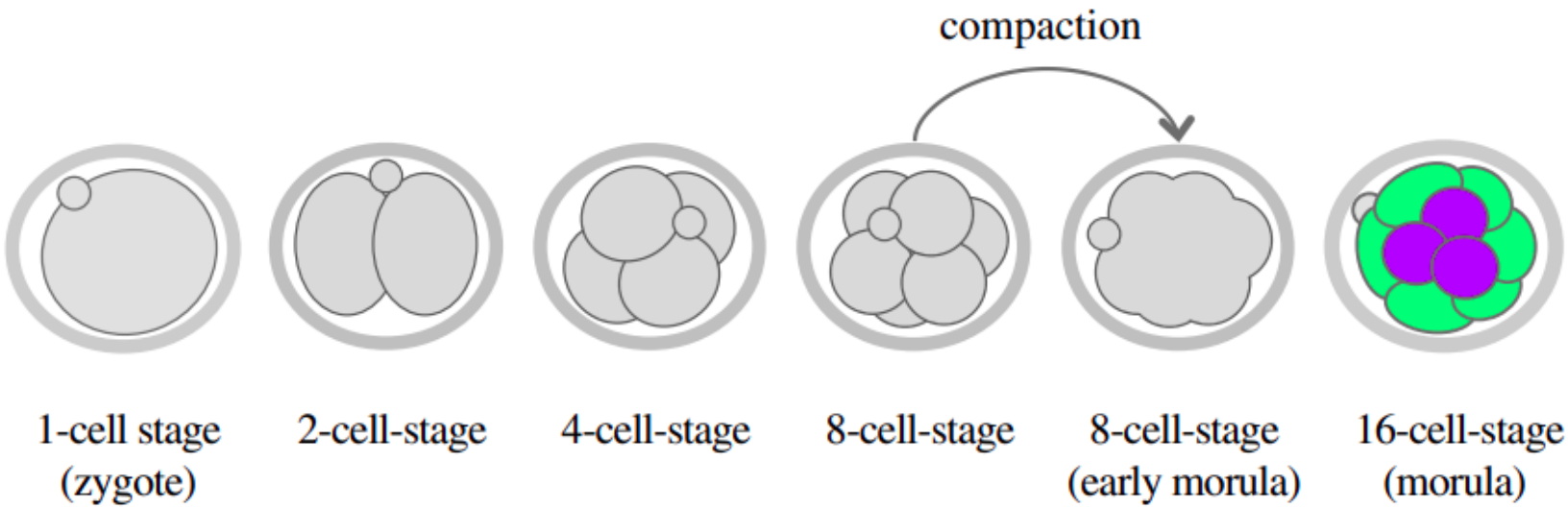


Mihajlovic et. al, *Open Biol.*, 2017.

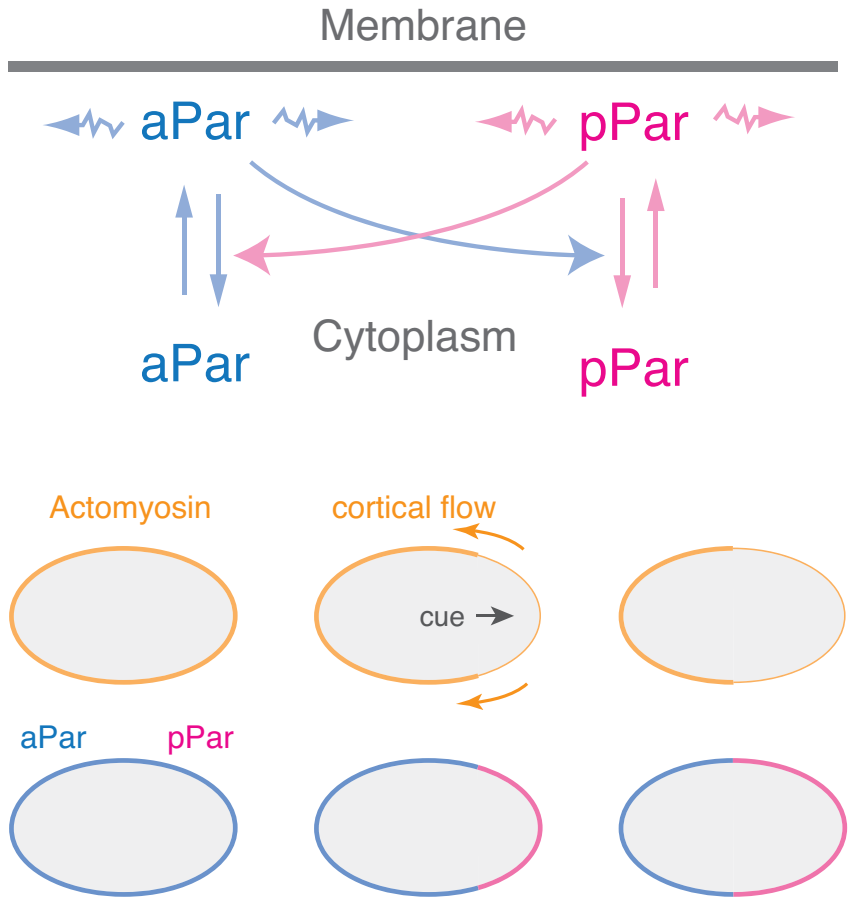
One-cell *C. elegans* model system

Ingredients

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

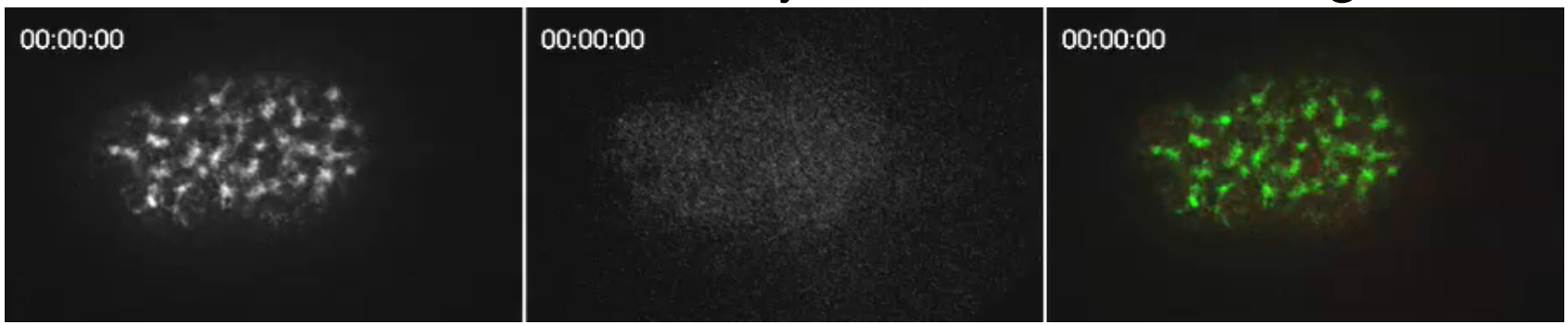
Wild type sequence

- Centrosomes $\rightarrow$ PAR-2 localized
- Sperm cue $\rightarrow$ Myosin inhibition
- Expansion of boundary to stable point ("establishment")
- "Maintenance:" boundary stays



Movie: *C. elegans* wild type

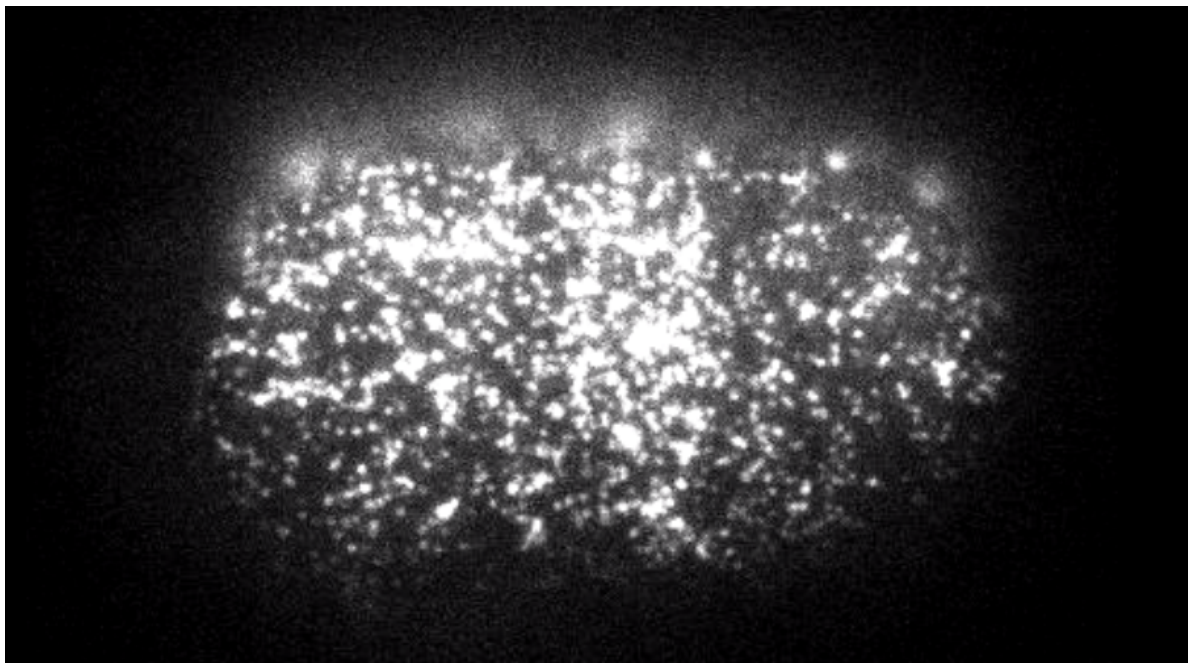
NMY-2::GFP | mCherry::PAR-2 | Merge



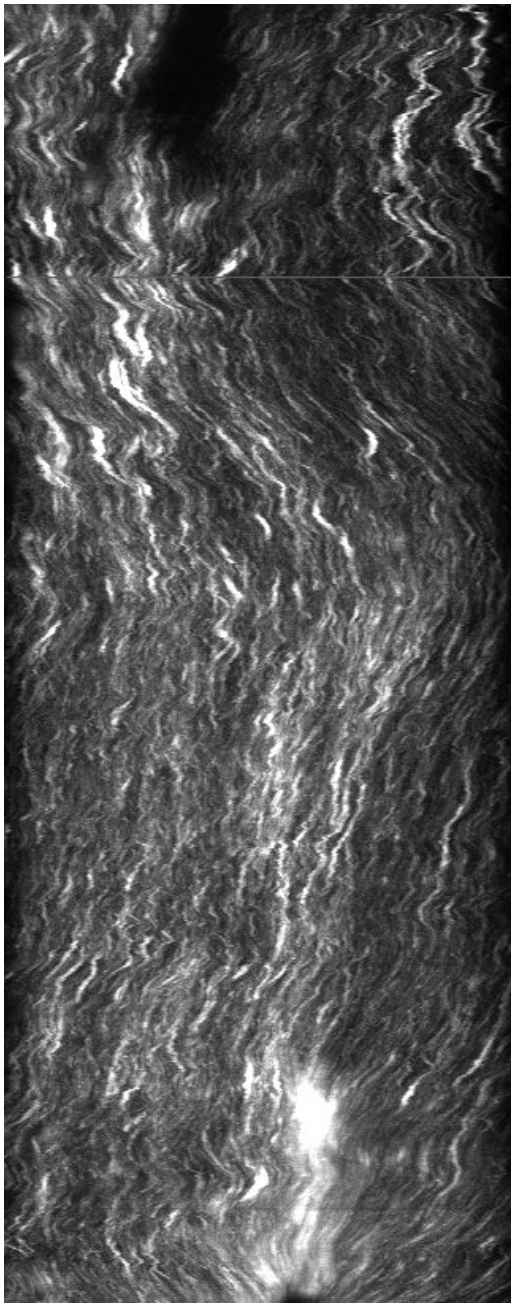
Zonies et al. *Development* (2010)

The wild-type boundary is stable (the movie)

Use CDK-1 (RNAi) to expand maintenance phase; boundary just sits there



The wild-type boundary is stable (the book)

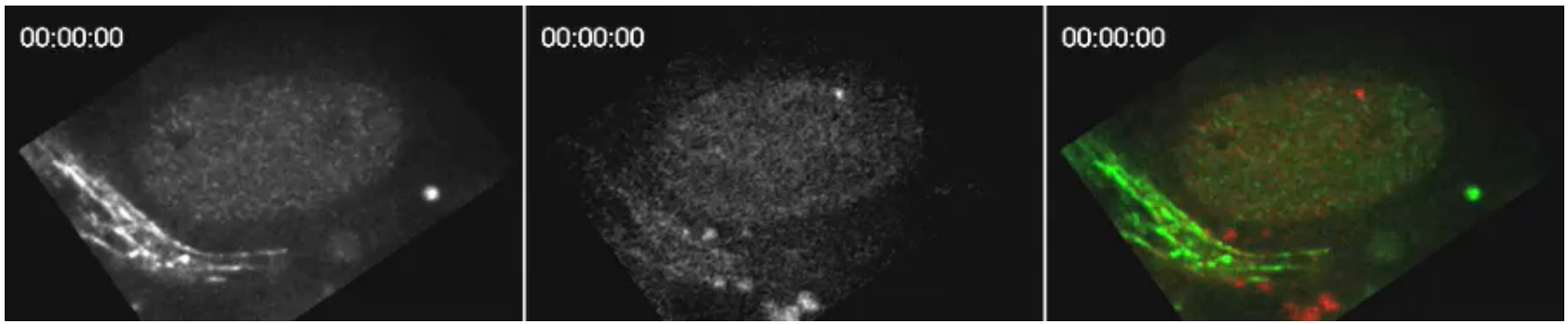


"Maintenance phase"

Does "maintenance" maintain the current boundary in non wild-type embryos?

- Establishment requires actomyosin
- Knockdown rho during establishment $\rightarrow$ no flows
- Alternative: ECT-2 knockdown (GEF that activates Rho)
- Both cases: local zone of PAR-2 enrichment remains

NMY-2::GFP mCherry::PAR-2 Merge



Results in exactly the same boundary position!

- Requires PAR-2, MRCK

Zonies et al. *Development* (2010)

The main questions

How do the aPARs (PAR-3), pPARs (PAR-2) and actomyosin flows combine to yield a dynamically stable boundary position?

- Hypothesis 1: maintenance phase "rescue" = actomyosin instability (self-patterning) + mutual inhibition of PARs
  - Uniform state is unstable
  - Fundamentally different from wild type
- Hypothesis 2: "rescue" = pPAR/aPAR competition + pPAR inhibiting myosin
  - Uniform state stable
  - Small asymmetry amplified
  - Same as establishment phase mechanism

Experimental and modeling program

Hypothesis 1: maintenance phase "rescue" = actomyosin instability (self-patterning) + mutual inhibition of PARs

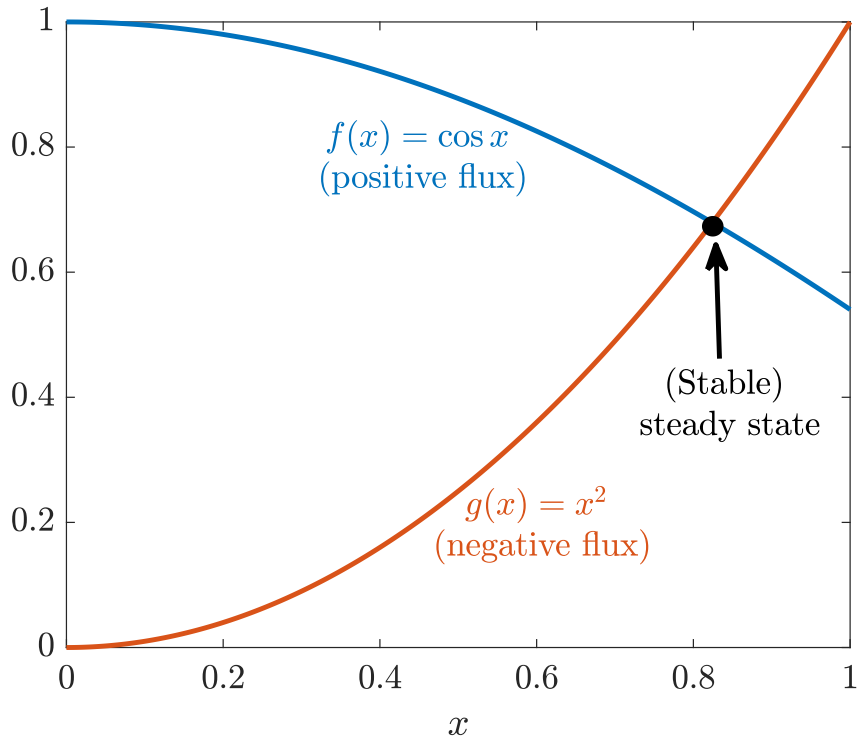
- Model myosin by itself
- Infer parameters from experiments
- Do we see self-amplification/instability?

Hypothesis 2: "rescue" = pPAR/aPAR competition + pPAR inhibiting myosin

Mathematics of stability

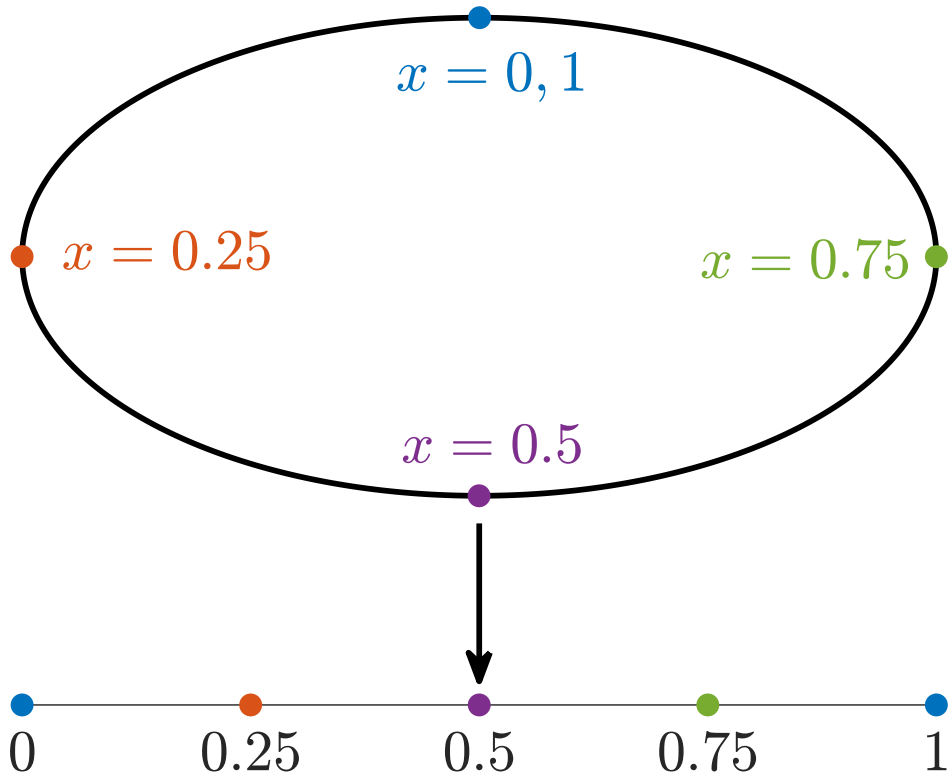
$$\frac{dx}{dt} = \underbrace{f(x)}_{\text{On rate}} - \underbrace{g(x)}_{\text{Off rate}}$$

- Steady states: $f(x_s) = g(x_s)$



- Stable steady state $f(x_s^+) < g(x_s^+) < 0$, $f(x_s^-) > g(x_s^-)$
- Perturb off the steady state and get pushed back to it

Model of the *C elegans* embryo



Myosin dynamics

$$\partial_t M + \underbrace{\partial_x (vM)}_{\text{Advection}} = \underbrace{D_M \partial_x^2 M}_{\text{Diffusion}} + \underbrace{k_M^{\text{on}} M_{\text{cyto}}}_{\text{On flux}} - \underbrace{k_M^{\text{off}} M}_{\text{Off flux}}$$

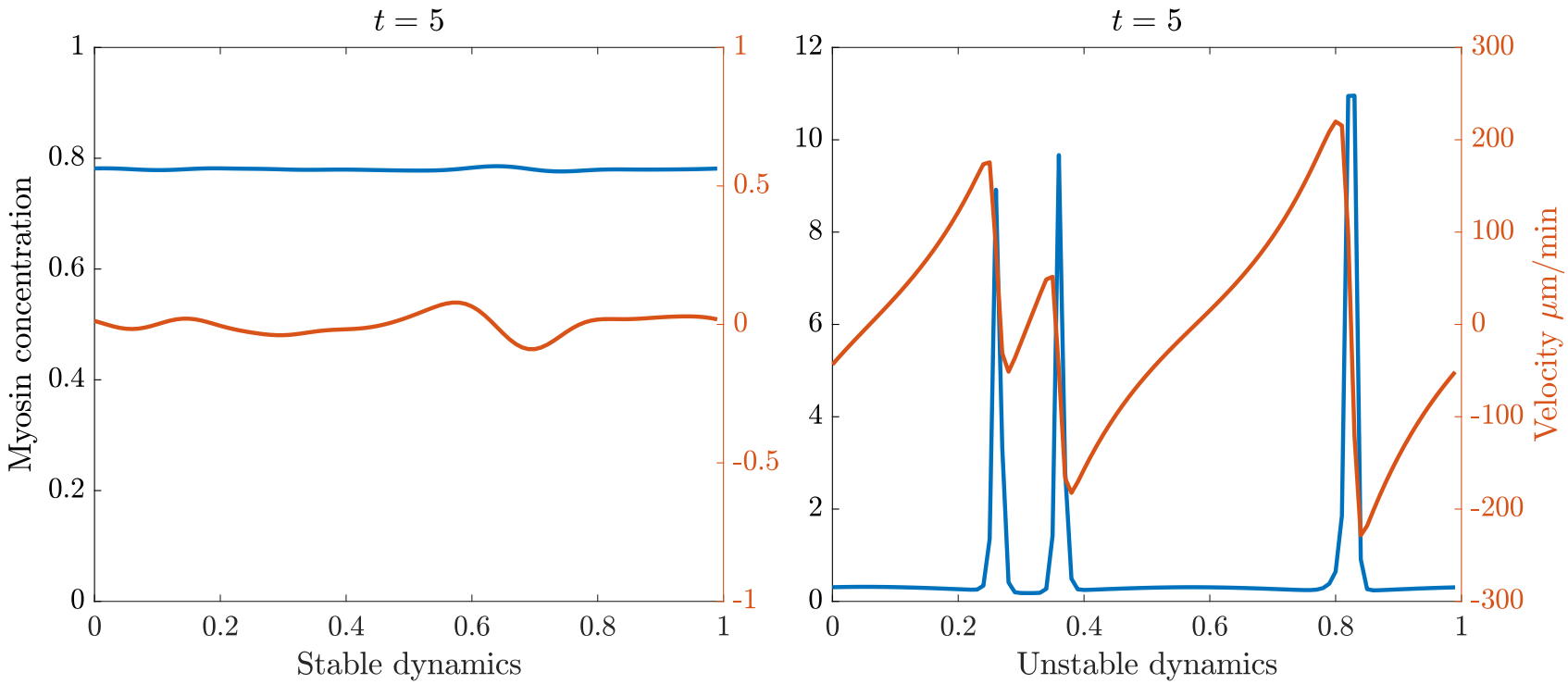
$$M_{\text{cyto}} = 1 - \int_0^1 M(x)\, dx$$

$$\underbrace{\gamma v}_{\text{Drag force}} = \underbrace{\eta \partial_x^2 v}_{\text{Viscous stress}} + \underbrace{\partial_x \sigma_a(M)}_{\text{Active stress}}$$

- Strong enough flows $\rightarrow$ self patterning
- Only unknown: stress vs. myosin relationship

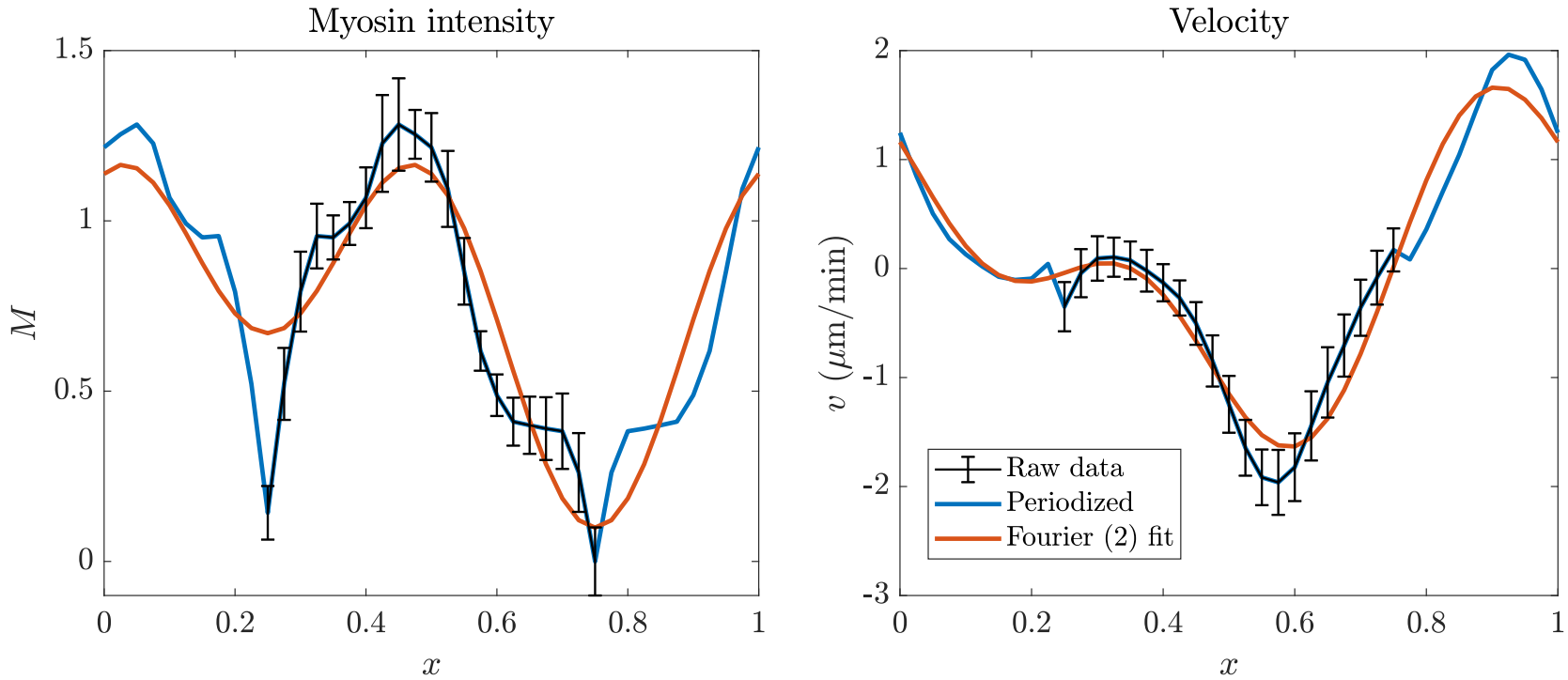
Bois et al. *PRL* (2011)

Stable and unstable myosin dynamics



Unstable flow patterns characterized by unphysical speeds

Inferred myosin velocity



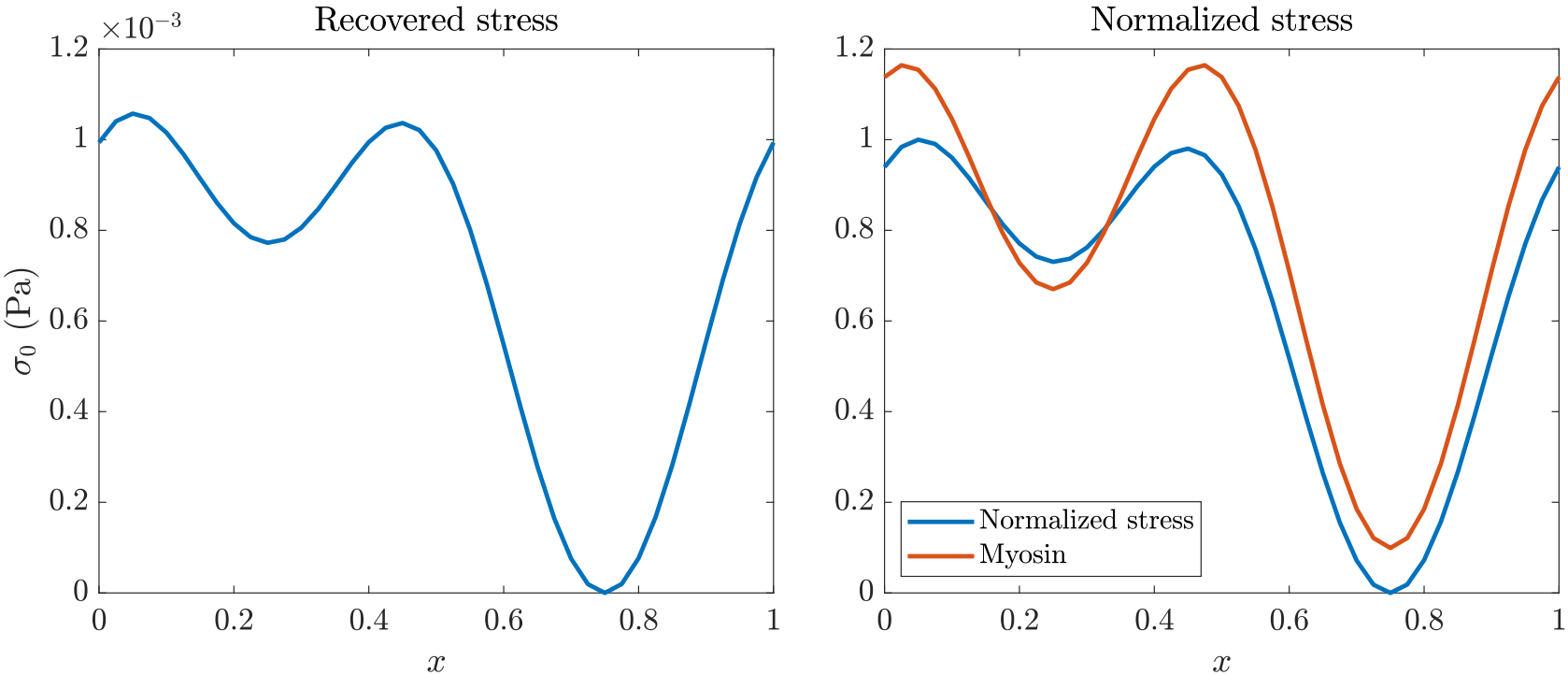
Solve

$$\gamma v = \eta \partial_x^2 v + \partial_x \sigma_a(M)$$

to get active stress $\sigma_a(M)$

Sailer et al. *Dev. Cell* (2015)

Inferred myosin dynamics



Active stress roughly proportional to myosin intensity

- $\sigma_a \approx \left(1.1 \times 10^{-3}\right) M$
- Not even close to strong enough for instability!
- Never see sharp peaks in arbitrary places
- Despite appearances, *no spontaneous symmetry breaking*
- System has 2 steady states

Experimental and modeling program

~~Hypothesis 1: maintenance phase "rescue" = actomyosin instability (self-patterning) + mutual inhibition of PARs~~

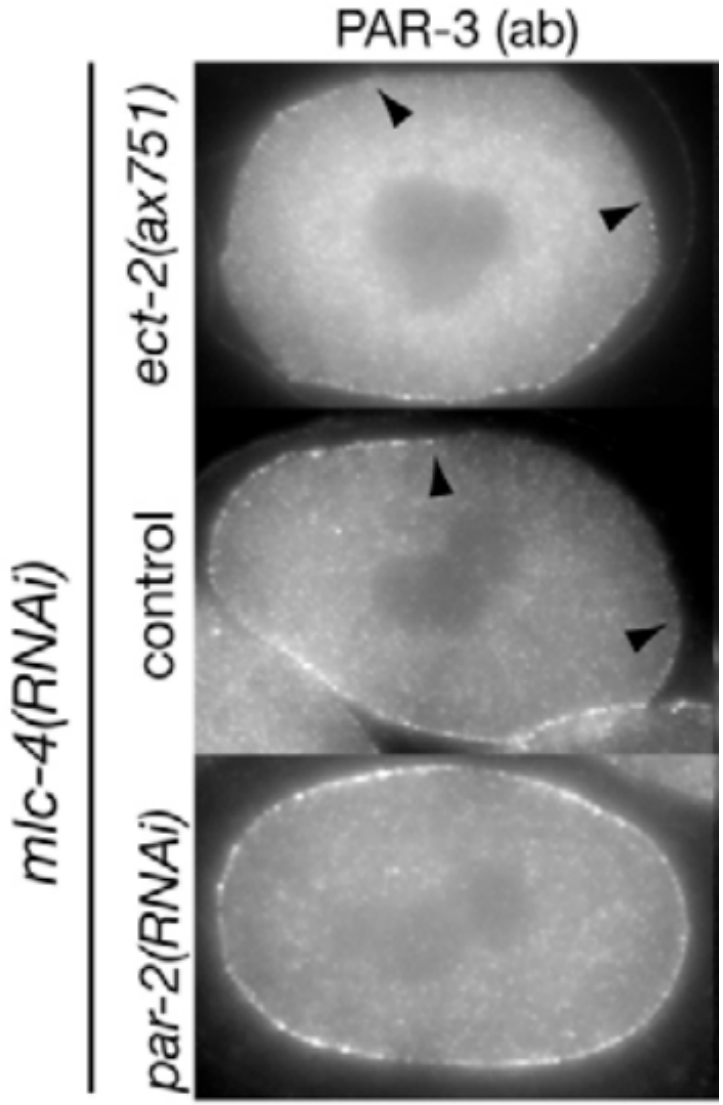
Hypothesis 2: "rescue" = pPAR/aPAR competition + pPAR inhibiting myosin

- Start with model of aPAR/pPAR competition
- Add myosin on top

PAR competition gives contracted boundary

PAR asymmetries stable by themselves without flows

- Wild-type embryos with MRCK knockout $\rightarrow$ boundary expands, but still asymmetric
- ECT-2 (RNAi) embryos: MLC-1 (RNAi) produces mutually inhibitory zones of PAR-2 and PAR-3
- Suggests that mutual antagonism encodes one steady state



Zonies et al. *Development* (2010)

First model for mutual inhibition

First model: mutual inhibition of proteins, mass-action kinetics

$$\partial_t A = D_P \partial_x^2 A + k_A^{\text{on}} A_{\text{cyto}} - k_A^{\text{off}} A - r_{\text{AP}} AP$$

$$\partial_t P = \underbrace{D_P \partial_x^2 P}_{\text{Diffusion}} + \underbrace{k_P^{\text{on}} P_{\text{cyto}} - k_P^{\text{off}} P}_{\text{Binding/unbinding}} \underbrace{- r_{\text{AP}} AP}_{\text{Mutual inhibition}}$$

- *One* stable steady state!
- Need a way to generate *bistability*

The Grill workaround

Polarization of PAR Proteins by Advective Triggering of a Pattern-Forming System

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In the *Caenorhabditis elegans* zygote, a conserved network of partitioning-defective (PAR) polarity proteins segregates into an anterior and a posterior domain, facilitated by flows of the cortical actomyosin meshwork. The physical mechanisms by which stable asymmetric PAR distributions arise from transient cortical flows remain unclear. We present evidence that PAR polarity arises from coupling of advective transport by the flowing cell cortex to a multistable PAR reaction-diffusion system. By inducing transient PAR segregation, advection serves as a mechanical trigger for the formation of a PAR pattern within an otherwise stably unpolarized system. We suggest that passive advective transport in an active and flowing material may be a general mechanism for mechanochemical pattern formation in developmental systems.

Developmental form emerges from coupling of pattern-forming biochemical networks with mechanical processes (*1*–*3*). In *Caenorhabditis elegans* zygotes, transient flows of a thin film of a mechanically active actomyosin cell cortex instruct the patterning of a conserved cell polarity pathway consisting of two groups of partitioning-defective (PAR) proteins that mutually exclude one another from the cell membrane (*4*–*14*). Initially, anterior PARs (aPARs: PAR-3, PAR-6, and atypical protein kinase C) cover the entire cell membrane. During polarization, flows of cortical actomyosin oriented away from the posterior-localized centrosome induces

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The Grill workaround

$$\partial_t A = D_A \partial_x^2 A - \partial_x(vA) + R_A$$

$$\partial_t P = D_P \partial_x^2 P - \partial_x(vP) + R_P \qquad (2)$$

where P is the local membrane concentration of pPARs, D_P denotes the diffusivity of membrane-bound pPARs, and R_A and R_P denote the reaction terms that describe membrane association and dissociation that now include interactions between the two species. We include reciprocal antagonistic feedback as detachment that depends on the concentration of the opposing species (Fig. 2A). Importantly, if this feedback is sufficiently nonlinear, mutual antagonism between A and P endows the system with a bistable character such that the membrane will tend to exist in one of two states: an anterior-like state, with $A > P$, or a posterior-like state with $A < P$ (see below) (*19*). Therefore,

$$R_A = k_{on,A} A_{cyto} - k_{off,A} A - k_{AP} P^{\alpha} A$$

$$R_P = k_{on,P} P_{cyto} - k_{off,P} P - k_{PA} A^{\beta} P \qquad (3)$$

where k_{AP} is a coefficient governing antagonism of A by P with α specifying stoichiometry and k_{PA} and β are similarly defined; $k_{on,P}$, $k_{off,P}$, and P_{cyto} are analogous to the corresponding A-specific variables. A similar framework without bistable character was proposed in (*20*), instead requiring local effects of the actin cortex on aPAR association rates for pattern formation.

Bistability in the reaction terms (Fig. 2B) has two important consequences that allow the system to account simultaneously for the unpolarized and polarized states of the embryo. First, bistability can drive the entire membrane of the system into one of two states: either an anterior-like homogeneous state, with aPARs enriched in the membrane and pPARs in the cytoplasm (similar to the *C. elegans* embryo before polarization), or the analogous posterior-like homogeneous state. Second, bistability also permits the system to support coexistence of distinct membrane domains in opposite states, separated in space and connected by boundary regions. A polarized system would contain exactly two such domains.

The Grill workaround

$$\partial_t A = D_A \partial_x^2 A - \partial_x(vA) + R_A$$

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2.3 Bistability

For $\alpha \geq 1$, $\beta > 1$ or $\alpha > 1$, $\beta \geq 1$, Eqns. 3 permit bistability in the reaction terms.

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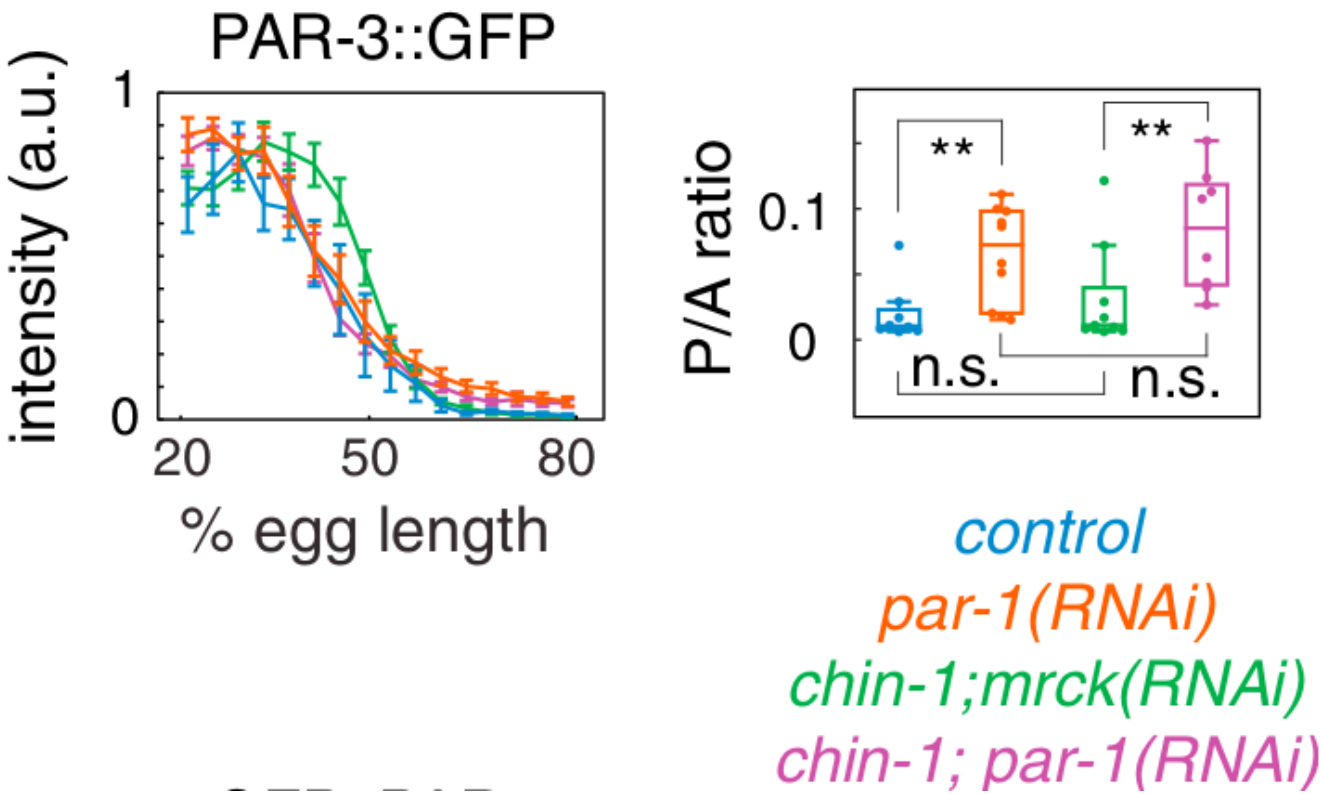
PAR-3 dynamics as a possible solution

Two important observations

- Without PAR-3, all other asymmetries lost
- pPAR (RNAi) $\rightarrow$ still get PAR-3 asymmetry
- PAR-3 must be the anchor for bistability in a sense



Sailer et al. *Dev. Cell* (2015)

Experiments → PAR-3 model

- Oligomerization
- Higher recruitment rate when more monomers attached
- *Feedback* model where bound monomers → more binding

$$\partial_t A_1 = D_A \partial_x^2 A_1 + \left(k_A^{\text{on}} + \underbrace{k_A^+ f_A^+ (A_{\text{tot}})}_{\text{Feedback}} \right) A_{\text{cyto}} + \underbrace{2k_A^{\text{dp}} A_n - 2k_A^{\text{p}} A_1^2}_{\text{Oligomerization}} - k_A^{\text{off}} A_1$$

$$\partial_t A_n = k_A^{\text{p}} A_1^2 - k_A^{\text{dp}} A_n$$

$$A_{\text{cyto}} = 1 - \int_0^1 A_{\text{tot}}(x)\, dx \qquad A_{\text{tot}} = A_1 + A_n$$

Feedback function

- Linear with saturation: $f_A^+(x) = \min(x, f_{\text{max}})$

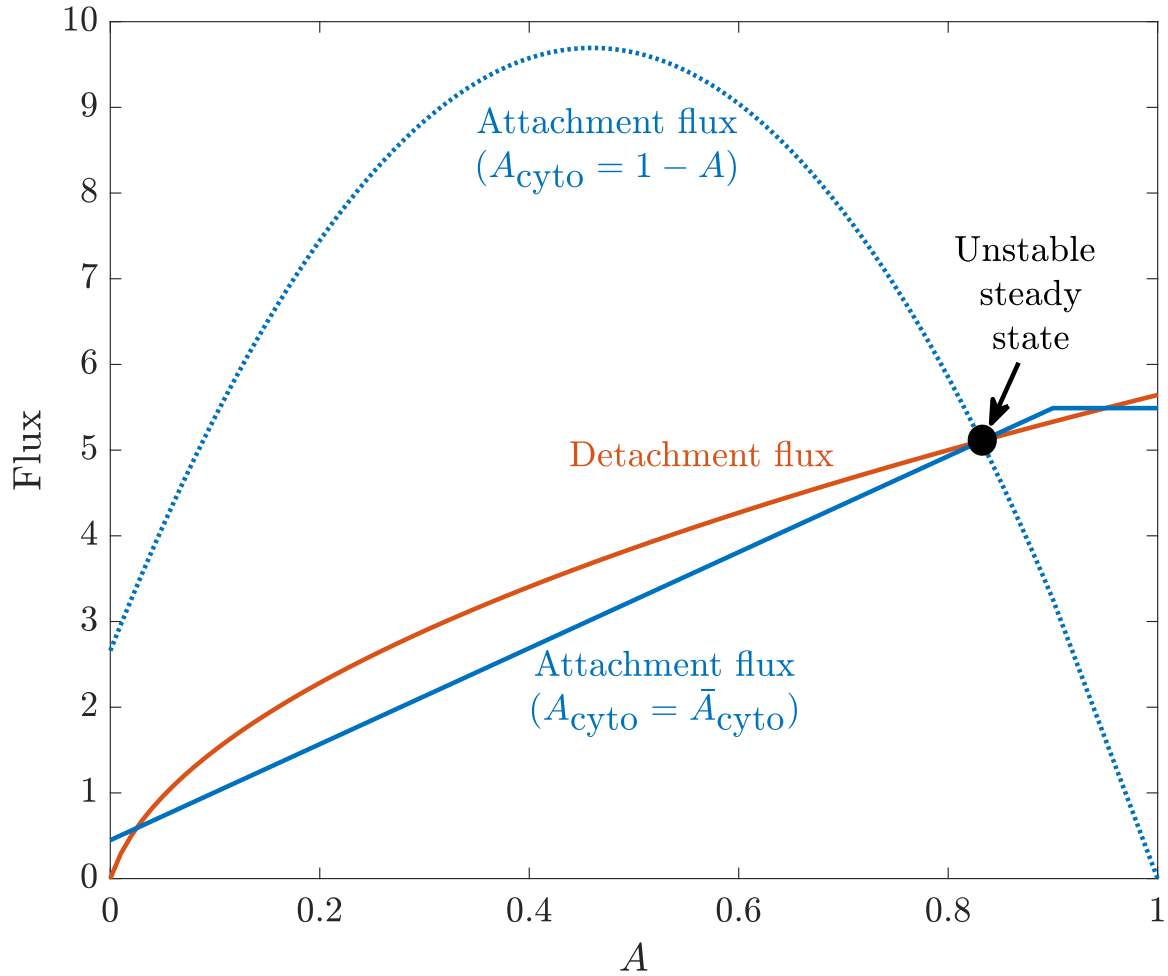
Cytoplasmic concentration

- $1 - A_{\text{tot}}$ at uniform steady state
- Constant when we do stability analysis

Lang and Munro. *Biophys. J.* (2022)

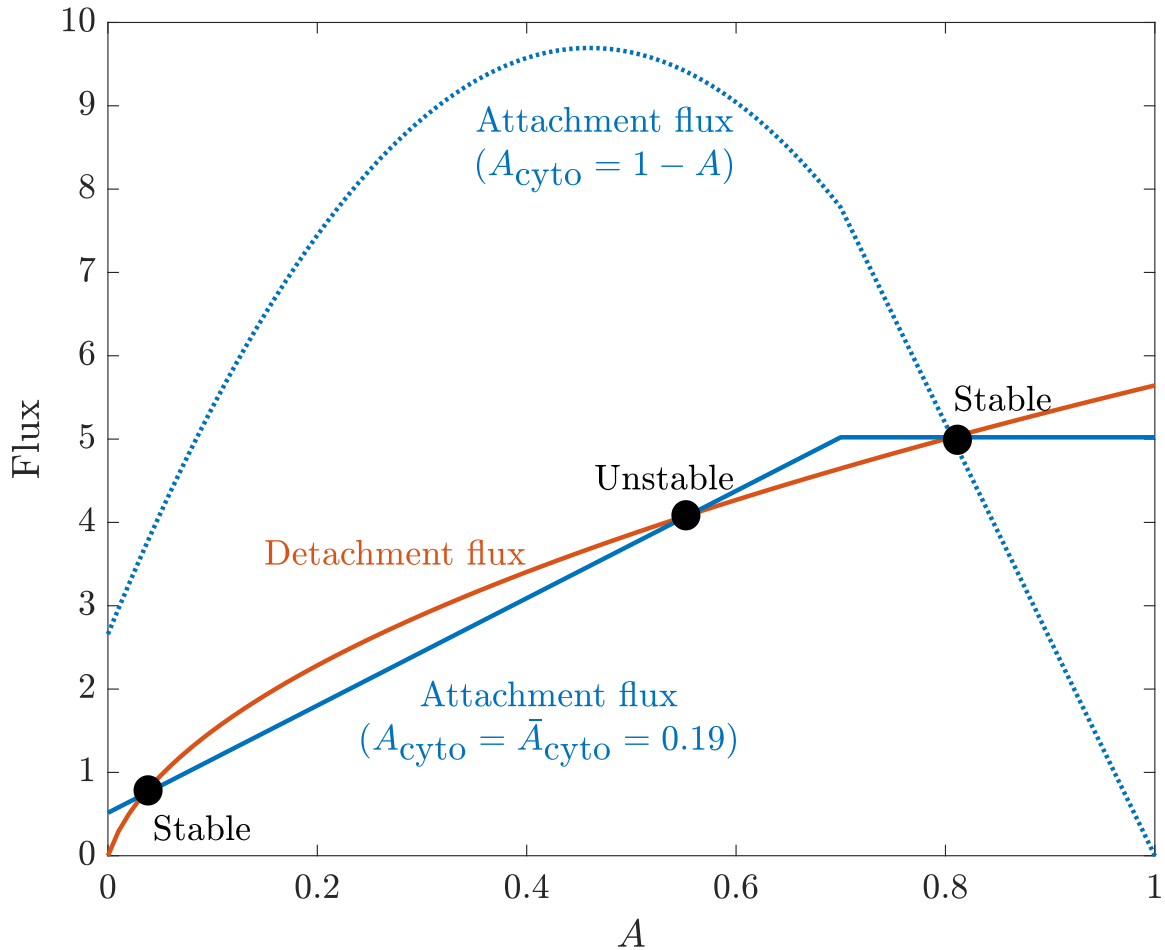
PAR-3 stability analysis

What happens when feedback strength is too large?



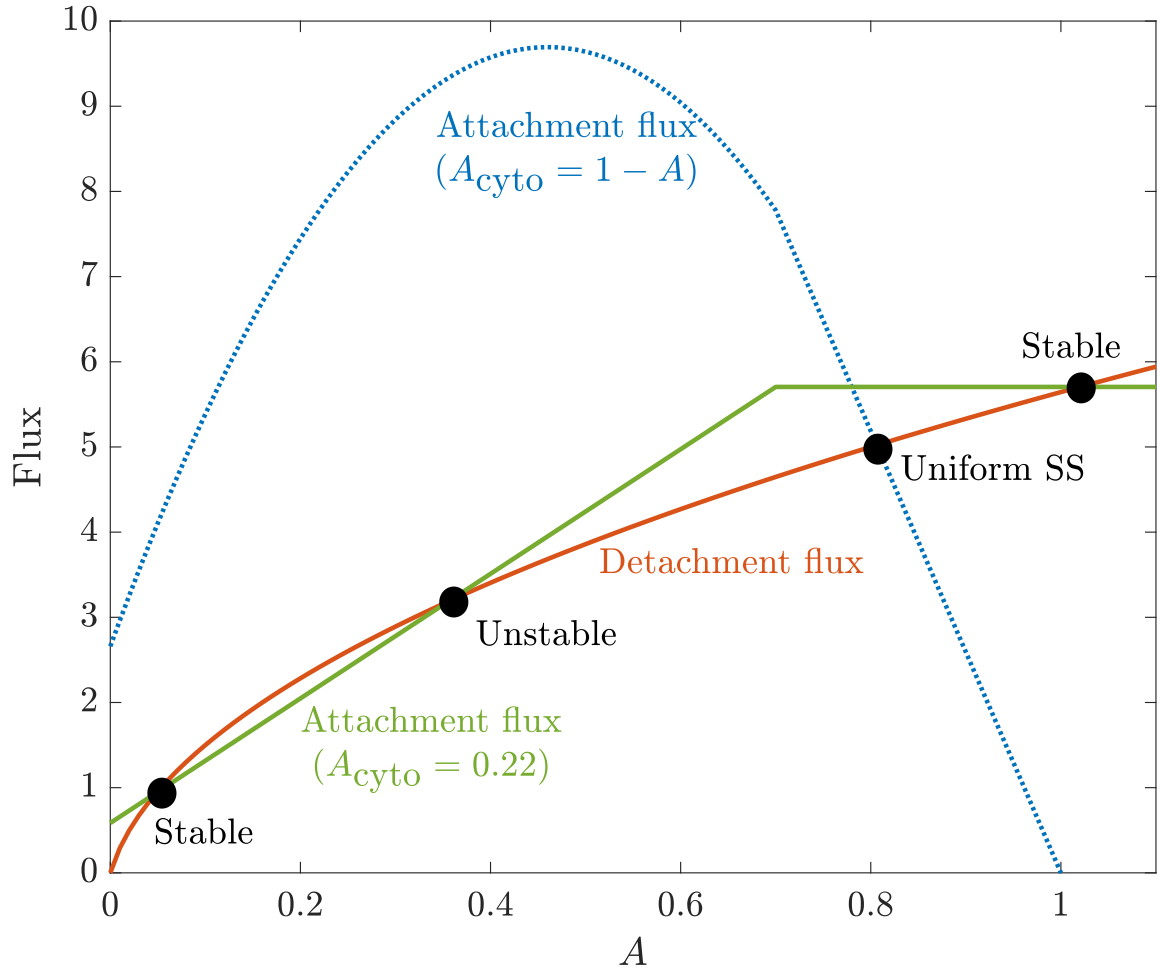
Smaller feedback saturation $\rightarrow$ stable uniform state

Bistability emerges at a fixed cytoplasmic concentration!



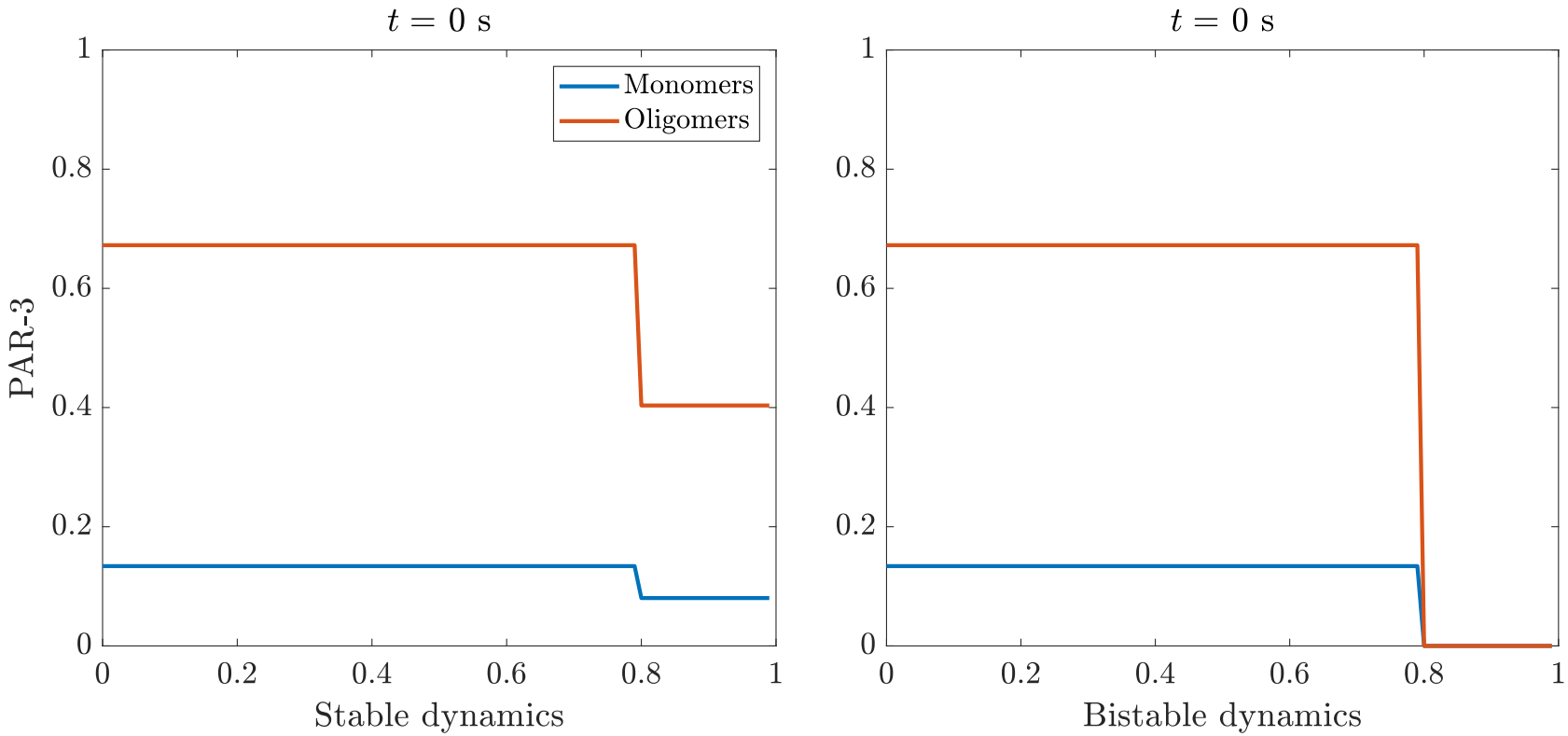
Cytoplasmic enrichment $\rightarrow$ larger steady states

Why polarized state is roughly 25% larger than uniform state



PAR-3 dynamics

Sufficient depletion of PAR-3 $\rightarrow$ bistability



Cytoplasmic enrichment $\rightarrow$ anterior state about 20% larger

Incorporating PAR-2

Introduce P (PAR-2/PAR-1 complex)

- Inhibits PAR-3 clusters
- Inhibited by PAR-3 (through PKC-3)

$$\partial_t A_1 = D_A \partial_x^2 A_1 + \left(k_A^{\text{on}} + k_A^+ f_A^+ \left(A_1 + 2A_n\right)\right) A_{\text{cyto}} - k_A^{\text{off}} A_1 - 2k_A^{\text{p}} A_1^2 + 2k_A^{\text{dp}} A_n,$$

$$\partial_t A_n = k_A^{\text{p}} A_1^2 - k_A^{\text{dp}} A_n - r_{\text{PA}} A_n P$$

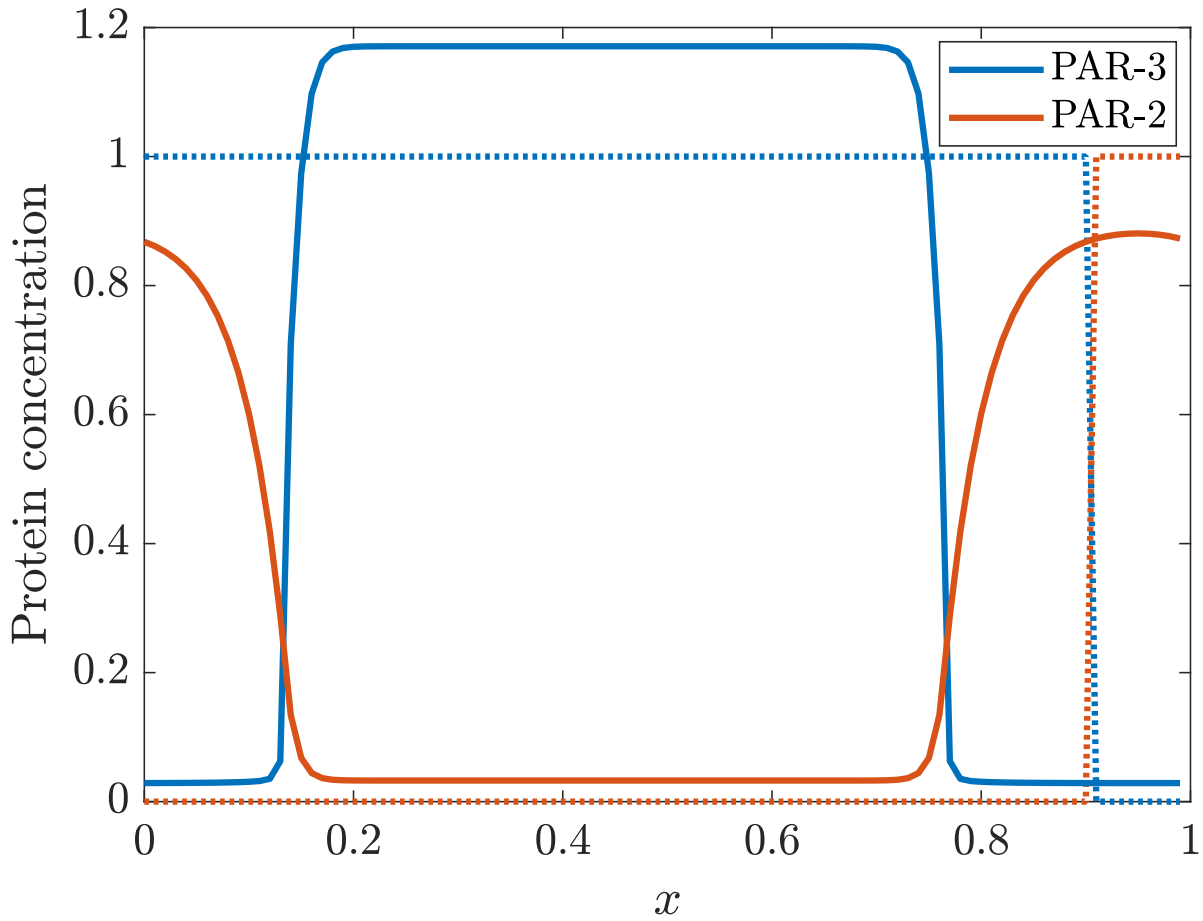
$$\partial_t P = D_P \partial_x^2 P + k_P^{\text{on}} P_{\text{cyto}} - k_P^{\text{off}} P - r_{\text{AP}} P \left(A_1 + 2A_n\right),$$

Tune parameter $r_{\text{AP}} = r_{\text{PA}}$ to match experiments ($\approx 70\%$ PAR-3 enrichment no myosin)

Lang and Munro. *Development* (2017)

Zonies et al. *Development* (2010)

Simulations with PAR-2



Initial domain naturally expands, then stops

- Lots of cytoplasmic PAR-2 $\rightarrow$ higher on flux
- Higher flux out-competes PAR-3, driving it lower
- Boundary stops when cytoplasmic PAR-2 is too low

Experimental and modeling program

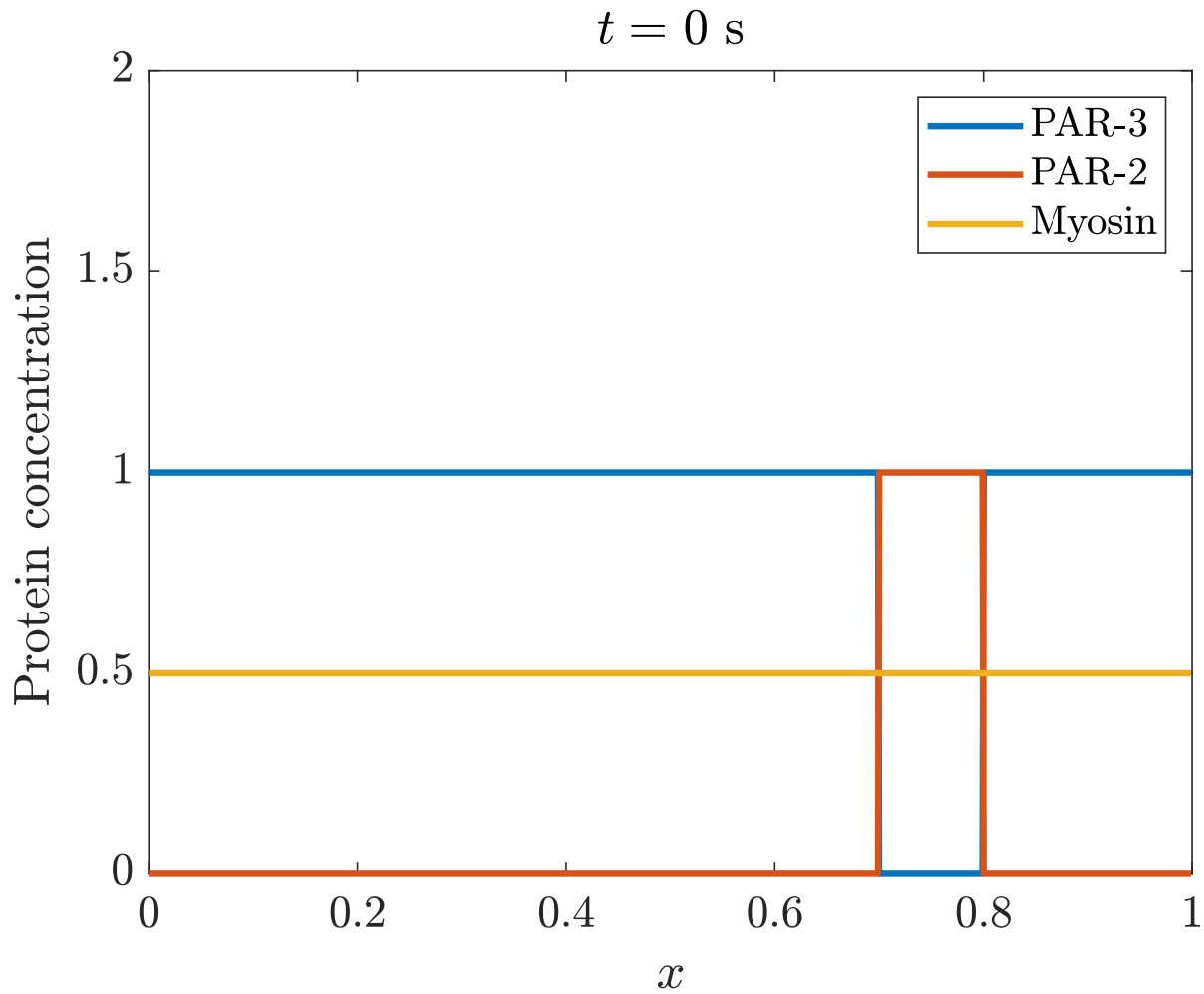
Hypothesis: "rescue" = pPAR/aPAR competition + pPAR inhibiting myosin

- Start with model of aPAR/pPAR competition
- Add myosin on top

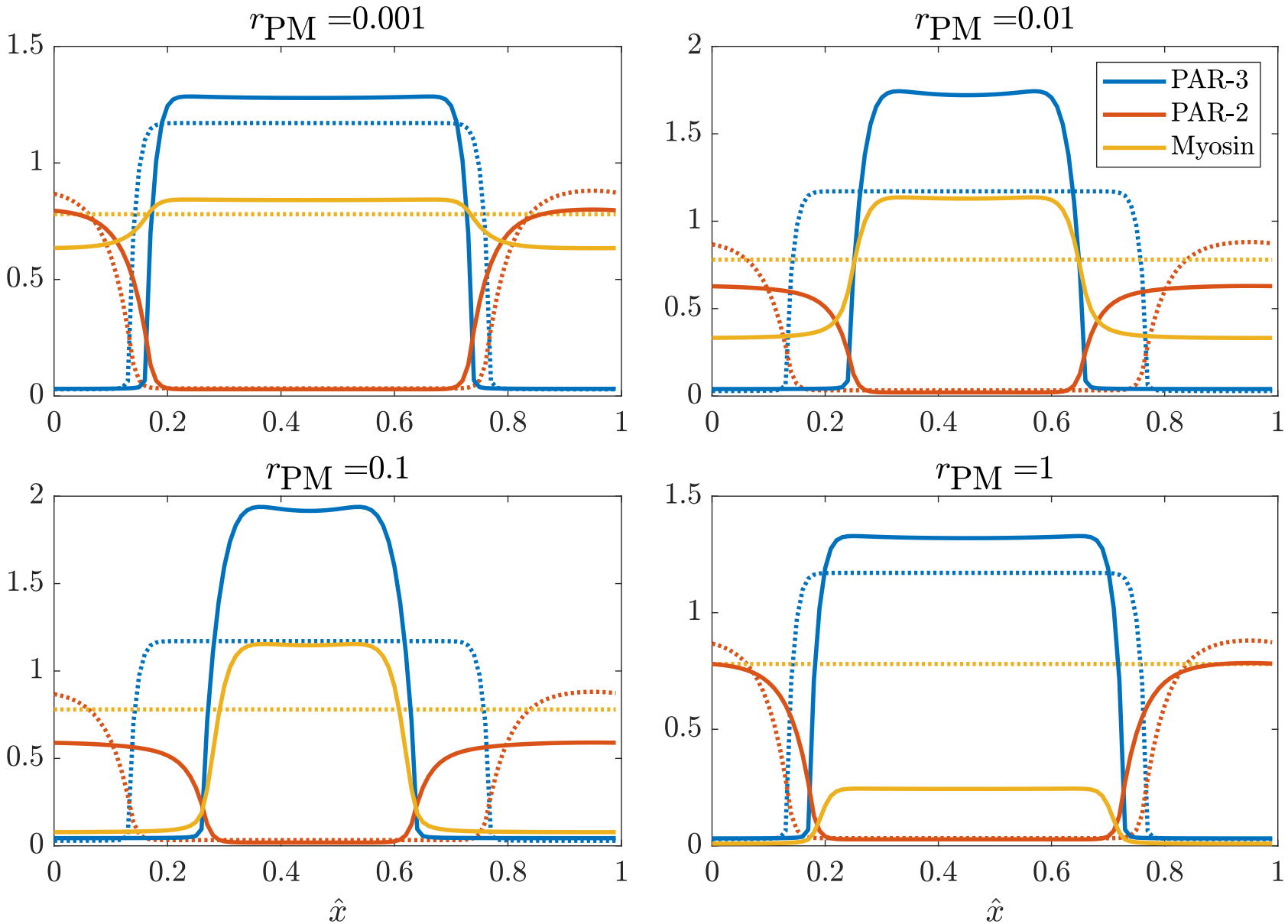
Full model: PAR-2/PAR-3/Myosin

Dynamics of myosin

- Inhibited locally by pPARs through mass-action kinetics
  $\partial_t M = \cdots - r_{\mathrm{PM}} MP$
- Transports everything (including itself!), $\partial_t P + \partial_x (Pv) = \ldots$

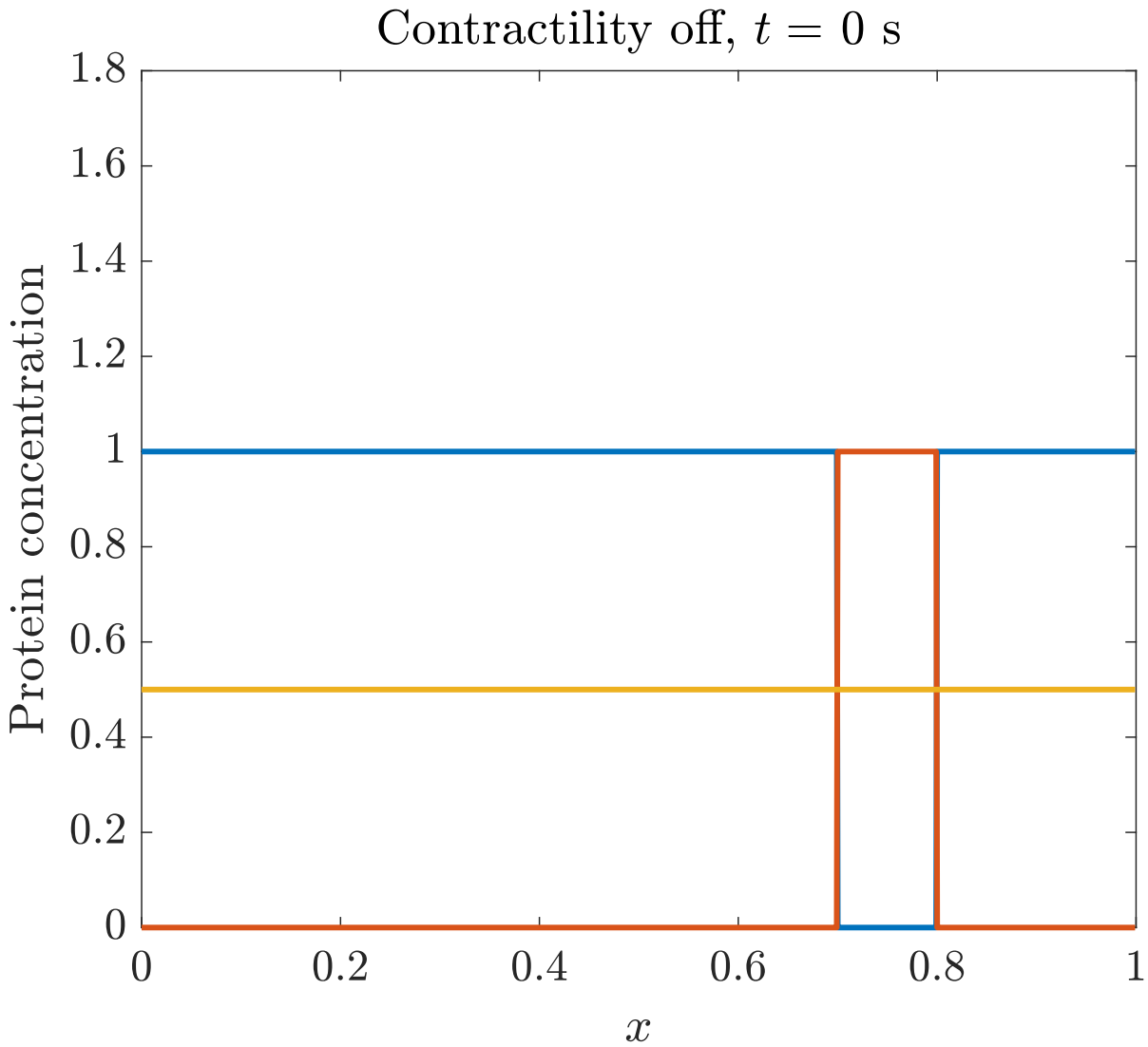


Steady states



Too or too much inhibition $\rightarrow$ no movement

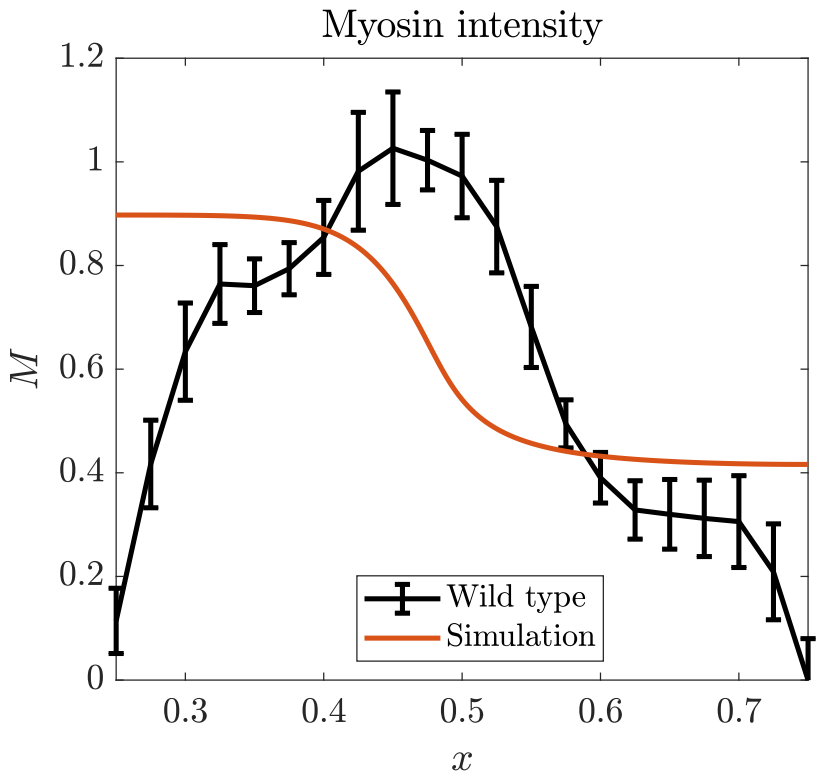
Contractility on/off

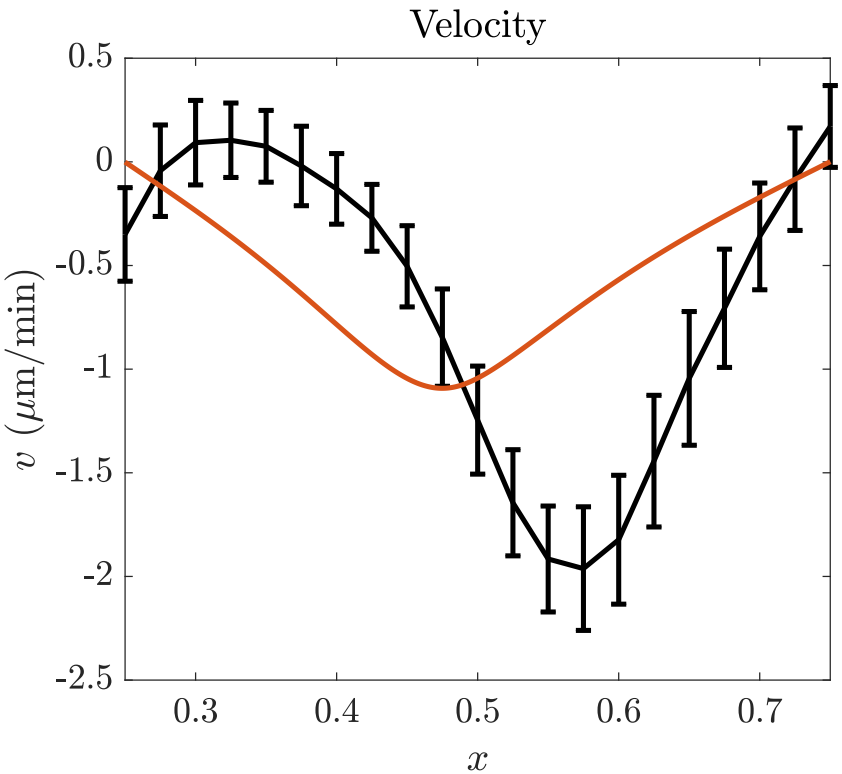


Remaining issues

What causes boundary to stop?

- Model: not enough cyto PAR-2 to outcompete PAR-3
- Experiment: something different?





- Strange myosin decrease towards anterior cap
- Concomitant "stall zone" of velocity

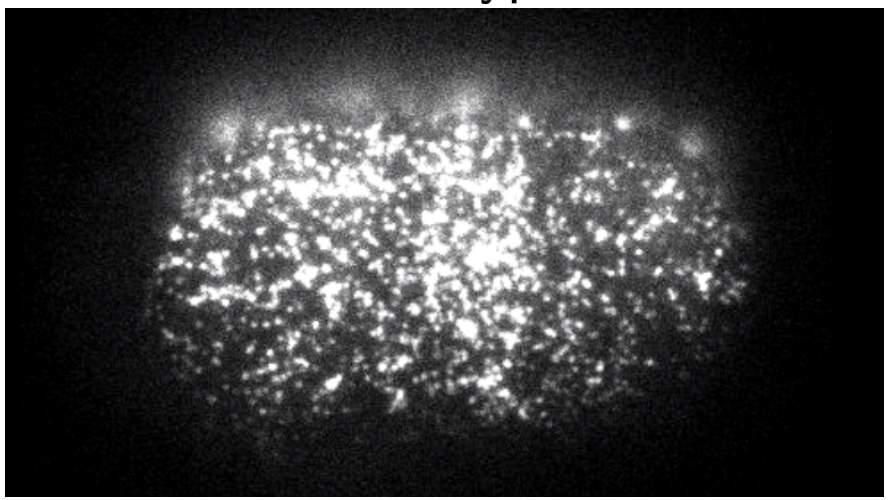
Sailer et al. *Dev. Cell* (2015)

Branched actin knockout movies

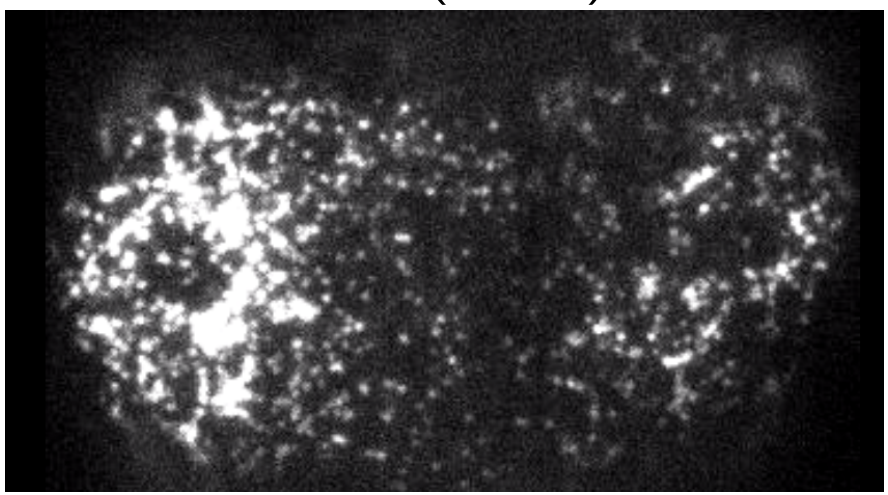
Issue: WT myosin peak sits at edge of anterior domain, not at cap

- Hypothesis: branched actin halts myosin constriction

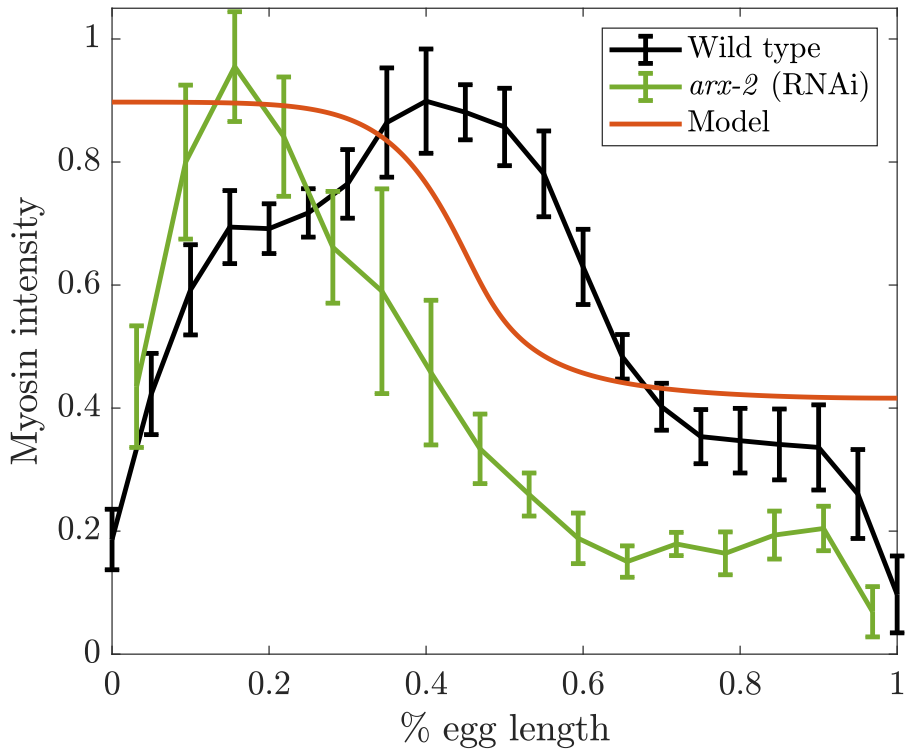
Wild type



arx-2 (RNAi)



Branched actin as a brake on contractility

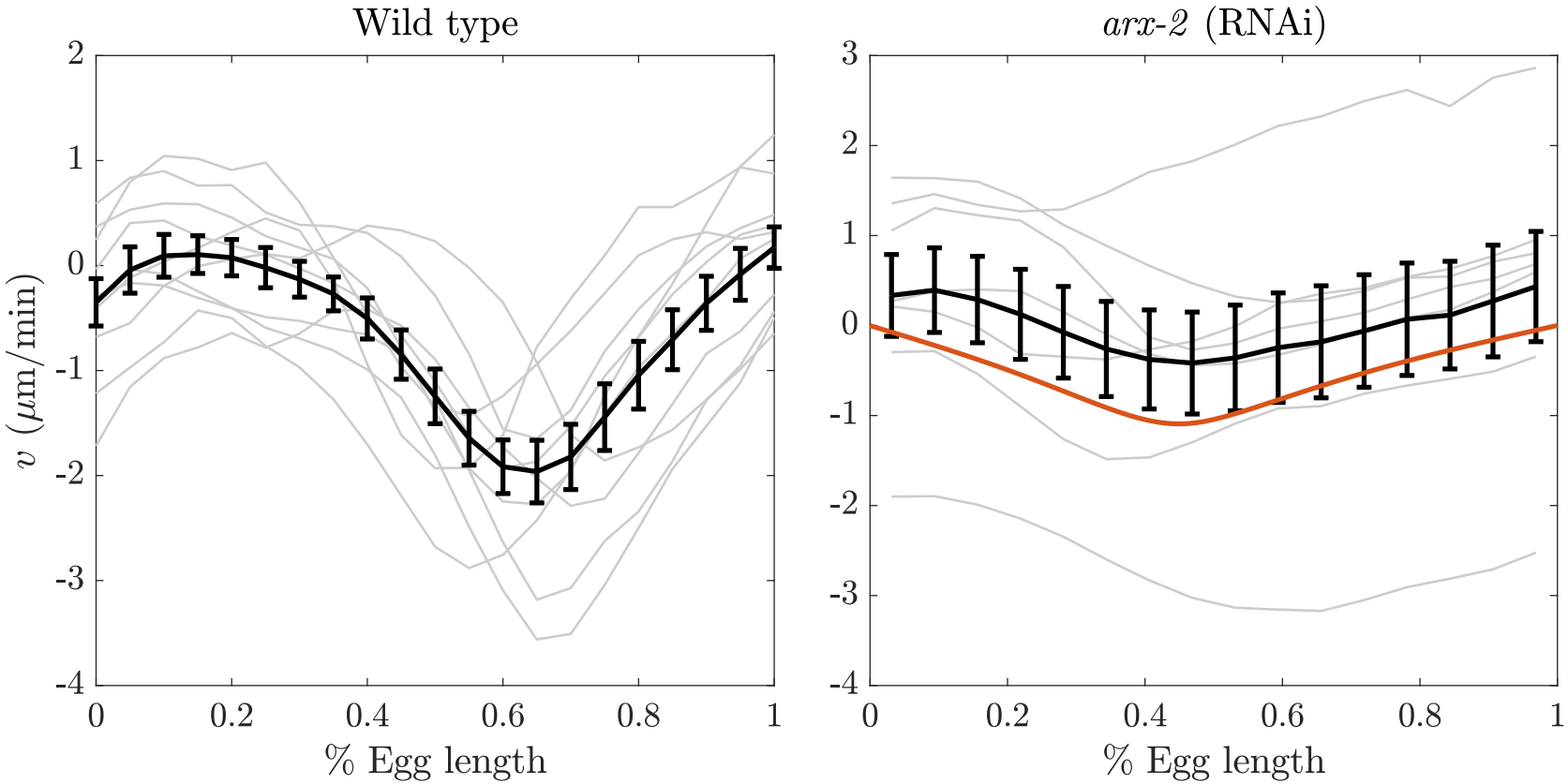


Myosin profile in *arx-2* (RNAi) looks similar to model

- Why decrease at boundary in *arx-2* (RNAi)? Imaging artifact?

Velocity field in *arx-2* (RNAi)

Wild deviations in *arx-2* embryos $\rightarrow$ hard to tell for sure



Appearance of flow profile that matches model

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
- Need better velocity field to confirm
- Branched actin inhibits contractility
- Incorporate into model; match WT?