

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

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## Introduction

Cell polarity is essential for many aspects of organismal development and physiology, including stem cell dynamics, directional cell migration, and asymmetric cell division (Dewey et al., 2015; Goldstein and Macara, 2007; Ierushalmi and Keren, 2021; Maître et al., 2016). For most cells, the first step in polarization involves a symmetry-breaking response to a transient localized cue that creates asymmetric distributions of specific molecules or molecular activities. The mechanisms that underlie cellular symmetry-breaking have been extensively explored (Li and Bowerman, 2010), but mechanisms that maintain polarity as a dynamically stable state with a fixed boundary position have only recently come under scrutiny (Sailer et al., 2015; Gross et al., 2019).

On a large scale, a cell’s polarity state is encoded by asymmetric distributions of protein molecules, which are shaped by smaller-scale processes like binding, diffusion, and active transport. In *C. elegans*, polarity is encoded by the distribution of two distinct groups of (highly conserved) polarity proteins: anterior PARs (aPARs), which include the scaffold PAR-3, adaptor PAR-6, atypical kinase PKC-3, and GTPase CDC-42, and posterior PARs (pPARs), which include the RING-domain containing protein PAR-2 (Bland et al., 2023), kinase PAR-1, tumor suppressor LGL-1, and CDC-42 GAP CHIN-1 (Lang and Munro, 2017).

Wild-type embryos polarize in two distinct phases termed “establishment” and “maintenance” (Cuenca et al., 2003). The mechanisms that underlie symmetry-breaking during polarity establishment have been well-studied (Cowan and Hyman, 2007; Munro and Bowerman, 2009). Near the end of meiosis, PAR-3, PAR-6 and PKC-3 are uniformly distributed at the cortex, where they prevent cortical association of PAR-1, PAR-2 and LGL-1 (Schonegg and Hyman, 2006; ?). One mode of symmetry-breaking involves the local inhibition of actomyosin contractility near the sperm MTOC,

which triggers cortical flows that transport F-actin, myosin II, PAR-3/PAR-6/PKC-3, and other cortically associated factors towards the anterior pole, resulting in their mutual restriction to an anterior cap (Munro et al., 2004; Rodriguez et al., 2017). Posterior depletion of PAR-3/PAR-6/PKC-3 then allows PAR-1, PAR-2 and LGL-1 to associate with a complementary posterior domain. Actomyosin contractility and cortical flow during polarity establishment require the small GTPase Rho-1 (Schonegg and Hyman, 2006; Motegi and Sugimoto, 2006), and in fact the sperm cue acts via Aurora A kinase (AIR-1) to locally inhibit Rho through the Rho GEF Ect-2 (Motegi and Sugimoto, 2006; Tse et al., 2012; Longhini and Glotzer, 2022; Kapoor and Kotak, 2019). A second mode of symmetry-breaking has also been described in which sperm astral microtubules promote local association of Par-2 with the posterior cortex (Motegi et al., 2011).

While the mechanisms that break symmetry are by now well understood, it remains unclear what *stops* symmetry breaking. If the system is programmed to amplify an initial polarizing cue, what sets the limit of amplification? The by now standard mechanism is that anterior (CDC-42/PAR-3/PAR-6/PKC-3) and posterior proteins (PAR-1/PAR-2/LGL-1) form a bistable reaction diffusion system by competing for residence at the cortex/plasma membrane such that one or the other but not both win locally. Theoretical studies suggest that in principle such a mechanism could stabilize the AP boundary after establishment phase cortical flows cease, or in embryos that lack cortical actin or myosin (Mori et al., 2008; Dawes and Munro, 2011; Goehring et al., 2011; Lang et al., 2023). These mechanisms by themselves cannot, however, shift the boundary on realistic timescales (Lang et al., 2023), which is why flow is required.

In the presence of flow, it is still unknown what sets a limit to boundary progression. Recent work showed that, assuming an intrinsic self limit to contractility, the boundary can be pinned, with the position set by total amount of each PAR protein (Gross et al., 2019; Goehring et al., 2011). But the self-limiting nature of the flow remains a mystery. In wild type embryos, myosin accumulates at the anterior in a cap that takes up about 50% of the embryo, and myosin flows from the posterior half of the embryo into the anterior cap. Despite the flow of myosin and the consequent A/P asymmetry in contractility, the cap maintains a fixed size (it does not contract), and the flow profile in the anterior is roughly zero (Sailer et al., 2015). Moreover, ectopic accumulation of Myosin II during maintenance in PAR-2 mutants is associated with abnormal posterior-directed cortical flows and rapid redistribution of Par proteins (Munro et al., 2004), suggesting that the distribution of myosin, in addition to the PAR proteins, guides the steady state.

While we will show that the self-limiting nature of contractility applies in both establishment

and maintenance phase, our focus in particular will be on maintenance, which requires CDC-42 to maintain asymmetries set up during establishment (Kay and Hunter, 2001; Gotta et al., 2001; Aceto et al., 2006; Schonegg and Hyman, 2006; Motegi and Sugimoto, 2006). GFP-tagged CDC-42 becomes anteriorly enriched during polarity establishment, and studies with a GFP-tagged biosensor suggest that the active (GTP-bound) form of CDC-42 may be similarly enriched during maintenance phase (Kumfer et al., 2010). Binding of CDC-42 to the conserved semi-Crib domain of PAR-6 is required for cortical association of PAR-6/PKC-3 during maintenance (Aceto et al., 2006), and PKC-3 is in an active state only when bound to CDC-42 (Sailer et al., 2015; Lang and Munro, 2017; Rodriguez et al., 2017). CDC-42 also acts through MRCK-1, a *C. elegans* orthologue of the mammalian Myotonia Dystrophy-related CDC-42-binding kinase MRCK, to promote asymmetric cortical recruitment of myosin II (Kumfer et al., 2010). There is also evidence that CDC-42 promotes asymmetric enrichment of F-actin during maintenance phase (REF?), but the underlying mechanism remains poorly understood. More generally, it remains unclear how these distinct outputs of CDC-42 are integrated to dynamically stabilize the AP boundary at a fixed axial position and limit contractility.

In this study, we combine experiments and theoretical modeling to show that boundary progression can be stalled when distinct outputs of CDC-42 have different effects on contractility. By extending both establishment and maintenance phases, we show that the boundary position is in fact stable, regardless of the cell cycle phase. We then demonstrate, through experiments with a temperature sensitive ECT-2 gene (Zonies et al., 2010), that the steady state observed at the end of maintenance phase can be reproduced even when establishment fails through a maintenance-phase rescue process, revealing that maintenance phase biochemistry encodes a dynamically stable attractive state. To understand how the PAR protein circuit (which involves CDC-42) interacts with myosin contractility to limit the extent of contractility, we introduce a continuum model. Without a self-limiting character to the flow, the boundary either contracts all the way to the end of the embryo, or slowly moves to the embryo interior over a timescale an order of magnitude longer than maintenance phase. To find the missing model component, we return to experiments, which demonstrate that branched actin in the anterior acts to reduce tension and prevent excessive contraction, similar to behavior observed previously in other systems (Muresan et al., 2022; Yang et al., 2012). Our model reveals that adding a threshold of CDC-42, above which branched actin is produced, is sufficient to reproduce the initial and latter stages of rescue, with the caveat that additional assumptions about branched actin are required to reproduce all experimental observations.

## Results

### Establishment and maintenance phase contractility are governed by unique biochemical systems

As a first step to examining the role of CDC-42 in regulating contractility, we sought to distinguish the phases of the cell cycle in terms of the upstream protein which ultimately regulates myosin. We began by imaging wild-type embryos expressing non-muscle myosin II fused to GFP (NMY-II::GFP), as well as embryos expressing PAR-6::GFP. At pseudo-cleavage and prior to the onset of centration (Fig. 1a), myosin exhibited pulsatile dynamics, with large foci appearing and disappearing over time, and PAR-6 was enriched in clusters on the anterior cortex (Fig. 1b). By nuclear envelope breakdown, both myosin and PAR-6 had transitioned to diffuse, smaller clusters enriched on the anterior cortex. Pulsatile myosin activity during establishment phase has previously been shown to involve rho-mediated contractility (Michaux et al., 2018; Michaud et al., 2022; Yao et al., 2022), while the diffuse myosin clusters have been previously linked to CDC-42 (Motegi and Sugimoto, 2006). Likewise, previous reports (Motegi and Sugimoto, 2006; Rodriguez et al., 2017) have shown that the diffuse PAR-6 clusters are correlated with binding to CDC-42, which activates PKC-3, while the punctate clusters are linked to PAR-3. We therefore conclude that Rho-1 mediates contractility during polarity establishment phase, while CDC-42 controls both contractility and PAR polarity during maintenance phase. The coupling of biochemistry and contractility through CDC-42 makes maintenance unique relative to establishment.

To understand the transition from establishment to maintenance, we depleted embryos of PRI-1 via RNAi. Depletion of PRI-1 prevented DNA replication and stalled cells in late establishment phase (Fig. 1c). In particular, we found a typical delay time of about eight minutes from pronuclear meeting to centration onset, relative to wild type embryos (Fig. 1d). The delay in centration onset also correlated with a delay in pseudo-cleavage relaxation (middle kymographs in Fig. 1c), and prolonged flows from the posterior into the psuedo-cleavage region (rightmost kymographs in Fig. 1c). Regardless of the embryo treatment, we found a strong correlation between three events: centration onset, psuedo-cleavage relaxation, and the myosin transition from punctate to diffuse. We conclude that these events mark the transition from establishment to maintenance, and especially to the regime of CDC-42-mediated contractility. While this regime is our interest here, it is noteworthy that embryos in late establishment phase (especially PRI-1 depleted embryos) exhibit a stable A/P myosin asymmetry characterized by persistent anterior-directed flow, similar

to previous observations for maintenance phase. We return to this point in the discussion.

## The steady state of maintenance phase is a stable boundary with persistent anterior-directed flow

It has been previously observed that embryos in late-maintenance phase exhibit a steady pattern of anterior-directed cortical flow (see (Sailer et al., 2015, Fig. 2)). Yet, despite the anterior-directed contractility, the A/P boundary in late maintenance appears to be stable. It remains unclear if this pattern of flow is a consequence of establishment-phase patterning of myosin (Munro et al., 2004), or if the pattern of flow is merely transient and would eventually stop if the cell cycle extended long enough. In this section, we establish that the pattern of flow and myosin profile in maintenance phase are in fact robust (to changes in establishment phase contractility) and long-lasting (if maintenance phase is extended); in other words, we seek to show that maintenance-phase biochemistry encodes an attractive steady state.

The pinning of the myosin boundary and subsequent decrease in the speed of flow hint that the cell has a built-in mechanism to prevent the boundary from contracting off the end of the embryo. But is it really possible to maintain a steady myosin intensity profile in the presence of a persistently anterior-directed flow?

To probe whether the myosin intensity and patterns of flow we see in late maintenance phase are truly steady states, we extend maintenance phase by treating wild-type embryos with CDK-1 (RNAi). Marking the start of maintenance phase by the end of pseudo-cleavage, and the end of maintenance phase by the onset of cytokinesis, we obtain a window of roughly 8–10 minutes per cell, in which we characterize the myosin intensity and flow speeds (see SI). Later stages of the extended maintenance show a relatively stable position of the myosin boundary (it in fact expands towards the posterior slightly) and a persistent anterior-directed flow which tends to achieve a maximum (magnitude) just posterior of the peak myosin location. Because the myosin intensity and flow speeds in *CDK-1* (RNAi) never resemble those of wild-type, it is clear that interfering with CDK-1 affects more than just the cell cycle, and it is impossible to say concretely that the specific flow and myosin profiles in late maintenance are “steady.” Nevertheless, we can say definitively that a persistent anterior-directed flow does *not* lead to further contraction of the boundary when maintenance is extended.

## The maintenance phase steady state is unique and attractive in the presence of MRCK

In addition to stabilizing asymmetries that are set up during establishment phase, the biochemical and mechanical interactions in maintenance phase can also rescue a steady polarized state. While this has been observed in previous studies in *ect-2* mutants (Zonies et al., 2010; Tse et al., 2012), which cannot activate Rho and consequently lack myosin functionality during establishment phase, it has yet to be shown that the steady polarized state that results is the same as in wild-type embryos. To establish this, we systematically image rescue of symmetry breaking in temperature-sensitive *ect-2* mutants, marking the beginning of maintenance phase via the transition of myosin from large to small clusters, and the end of maintenance phase as the onset of embryo rotation prior to cytokinesis. This gives a roughly four minute window of maintenance phase for each embryo, in which we track the average myosin intensity profile and flow speed.

### Existing models cannot explain the dynamics of maintenance phase rescue

Our goal in this section is to explore existing literature mechanisms for how maintenance phase biochemistry could set an attractive steady state. At first glance, it appears that the phenomenon of maintenance phase rescue is a consequence of an instability of the underlying system; a small perturbation in the myosin concentration could induce an anterior-directed flow, which drives the system to the polarized state. Here we systematically exclude this by considering a model of myosin alone and showing that spontaneous symmetry breaking is not possible with realistic parameters. We then consider PAR proteins, showing that, while the PAR circuit we consider can maintain a stable polarized state with a fixed boundary position, the time required to set up this state is prohibitively long, and myosin-directed flows are required. Finally, we consider a combination of myosin-driven flows *and* PAR proteins. This model allows us to reproduce the initial stages of maintenance phase rescue, but it cannot explain the sudden slow-down in flow speeds and pinning of the myosin boundary.

In the case when diffusion of myosin is negligible and all myosin is bound to the membrane, the stability analysis in the SI shows that the dynamics are unstable when the flow carries myosin molecules a distance larger than the hydrodynamic lengthscale, i.e.,  $v/k_M^{\text{off}} > \ell$ . Substituting  $\ell \approx 14 \mu\text{m}$ , we plot the velocity threshold for instability as a function of myosin lifetime in Fig. S4. For realistic lifetimes on the order 5–20 s, the minimum flow speed to generate instability is about

$40 \mu\text{m}/\text{min}$ , which is much faster than ever observed *in vivo*. Thus the dynamics of maintenance phase rescue are not due to myosin instabilities.<sup>1</sup>

Because the dynamics of myosin alone are insufficient to generate instability, PAR proteins must be essential for rescue to occur (Zonies et al., 2010). We consequently introduce a model of maintenance phase biochemistry based on the diagram in Fig. ??, which in turn comes from (Lang and Munro, 2017, Fig. 2). On the anterior side, we have three distinct protein species: PAR-3 ( $A$ ), CDC-42 ( $C$ ), and PAR-6/PKC-3 ( $K$ ), each of which has a separate function. The posterior PARs (PAR-2, PAR-1, and CHIN-1) can be lumped into one species (denoted by  $P$ ), which antagonizes both PAR-3 and CDC-42. The equations which describe the circuit are given in the SI.

In previous work on PAR-3 (Lang et al., 2023), we demonstrated that the diffusion of the smaller oligomer sizes (monomers in our model) sets a unique boundary position. In Fig. ??, we demonstrate that this with the full biochemistry by simulating the contraction of a PAR-3 domain that initially takes up 90% of the embryo. The boundary shifts because a high concentration of pPARs shifts the local equilibrium of PAR-3 oligomerization towards the monomer state, which makes bistability impossible (Lang et al., 2023). Consequently, we observe contraction of the PAR-3 domain, with a peak that grows over time, and expansion of the PAR-2 domain, both of which eventually reach a steady state. The concentration of PAR-2 at the edge of the domain decreases over time, which suggests that cytoplasmic depletion might be responsible for pinning the boundary. To demonstrate that a fixed pool of protein is key to arresting posterior domain expansion, in the right panel of Fig. ?? we simulate with a cytoplasmic pool that is “frozen” at its value at  $t = 10$  mins. The result is an anterior domain which shrinks at a constant rate with constant peak concentration, and a posterior domain which expands at a constant rate with the same concentration at the edge. Given enough time, the posterior domain invades the entire embryo length.

In (Lang et al., 2023), we showed that the diffusion-controlled shift of the PAR-3 boundary occurs on timescales of hours, meaning that the boundary never reaches a steady position in practice. This is confirmed in Fig. ??, which shows that cytoplasmic depletion can only act rapidly to stop boundary progression if the initial boundary size is close to the steady state. The simulation in Fig. ??(a), which starts at 90% PAR-3 enrichment takes some two hours to reach the steady state; while a simulation starting at 50% PAR-3 enrichment takes only 5–10 minutes. In order to

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<sup>1</sup>We note a distinction here between maintenance and establishment phase. In the latter, instabilities in the dynamics of rho combine with delayed negative feedback to yield pulsatile dynamics (Nishikawa et al., 2017; Michaux et al., 2018; Michaud et al., 2022). The connection between these pulsatile dynamics and the large-scale flows that establish polarity is still unknown.

reproduce rescue, we need to introduce cortical flows that initially shift the boundary at a faster initial rate. If these cortical flows are then arrested, cytoplasmic depletion can then prevent the boundary from shifting, leading to a steady state.

Because neither myosins nor PAR proteins can rescue maintenance phase asymmetries by themselves, there must be an interaction with PAR proteins that amplifies gradients in contractility to rescue the correct polarized state. To account for this, we add the myosin dynamics (??) to the biochemistry system (??). In doing this, we also incorporate advective terms that ensure that each protein moves with the local cortical velocity (Illukkumbura et al., 2023), and make CDC-42 a promoter of myosin. The equations corresponding to this situation are a straightforward extension of (??) and (??), and we therefore confine them to the supplemental material, where we give a non-dimensional version and fit the unknown parameters.

In Figure ?? shows the results when we the parameters to match the experimentally-measured initial speeds of rescue. The left panel shows the dynamics of the simulation from  $t = 0$  (lightest colors) to  $t = 7.5$  mins (darkest colors), while the right panel shows the myosin boundary position (0 is the anterior pole, 1 is the posterior pole) and the flow speeds over time. Our statistics show the beginning of the rescue process, where an initially peaked profile of PAR-2 invades the anterior domain, concentrating anterior PARs in the middle and thereby increasing the concentration of pPARs in the posterior. As a result of this, CDC-42 gets inhibited in the posterior, which gives a gradient of myosin from posterior to anterior. The gradient of myosin generates a flow which further compacts the anterior domain. The timescale of this compaction is much faster than without flows (Fig. ??), and indeed occurs on a timescale of minutes and not hours.

While our model reproduces the initial stages of the rescue process, there is no mechanism in it to halt the advancing myosin front. With the correct model parameters, the tendency of flow to concentrate the aPARs overwhelms the effect of cytoplasmic depletion (which tends to slow the boundary motion). As a result, the boundary contracts with nothing to slow it down. Indeed, in the limit as time goes to infinity, we expect one peak of aPARs and myosin at the anterior pole, which is in sharp contrast to our experimental result which showed a sharp drop in the anterior-directed flow speed in the late stages of maintenance phase (see inset of Fig. ??).

### Branched actin prevents hypercontractility and is necessary for successful rescue

Our modeling so far identified two regimes of behavior, depending on the sensitivity of myosin to the CDC-42 concentration. Roughly speaking, if CDC-42 promotes myosin at a rate much smaller

than the basal rate (0 in Fig. ??), the cytoplasmic dynamics are sufficient to stop the pPARs from invading too far into the anterior domain. But this model has to be discarded because the dynamics occur over unrealistically long timescales. To match the speed of rescue, CDC-42 has to promote myosin at a rate much larger than the basal rate, which leads to fast flows and (in this model) a rapid concentration of the anterior domain into a peaked profile at the anterior pole (Fig. ??). Thus, we are missing an important interaction that slows down the advancing myosin peak, rapidly dropping the flow speed and allowing cytoplasmic depletion to pin the boundary. This interaction is the focus of this section.

In Fig. ?? we show the myosin intensity and flow speed over 30 second intervals of maintenance phase rescue, and compare the result to the steady myosin intensities and flow speeds observed in wild-type embryos (Sailer et al., 2015). The myosin peak initially grows in size advances rapidly towards the anterior, but after about three minutes it stops growing and becomes pinned at about 40% embryo length. The pinning of the myosin peak corresponds to a decrease in the maximum flow speed from about  $4.5 \mu\text{m}/\text{min}$  (from 2:00 to 3:30) to  $2 \mu\text{m}/\text{min}$  in the last 30 seconds of maintenance phase (3:30 to 4:00, see the inset of Fig. ??). In the last 30 seconds of maintenance phase, the flow profile in *ts ect-2* embryos is roughly the same as that observed during late maintenance phase in wild-type embryos. The myosin intensity appears quantitatively different, but has the same fundamental shape: there is a gradient of myosin in both the anterior and posterior, with a maximum occurring at around 40% of the embryo length. These results establish that the biochemical circuit governing maintenance phase can amplify small residual asymmetries from establishment phase, and that the resulting polarized state is independent of what comes before maintenance.

We hypothesize that branched actin might stop the anterior cap from becoming hyper-contractile. To test this hypothesis, we deplete wild type embryos of *arx-2*, a protein which activates the arp 2/3 complex and the assembly of branched actin. Figure ?? shows the average myosin intensity and flow profiles over the last three minutes of maintenance phase in wild-type (red) and *arx-2* (RNAi) treated embryos (blue). While the wild-type intensity seems to be roughly stalled at a boundary position 40% of the embryo length, the *arx-2* (RNAi) embryos demonstrate hyper-contractility, with a myosin peak that shifts to the anterior (and increases) over time.

The flow measurements in *arx-2* (RNAi) embryos are chaotic and noisy because the hyper-contractile anterior cap is not always positioned directly on the anterior pole, so flows can be generated off the A/P axis. In Fig. ??, we show the average flow speeds in embryos where the

A/P flow dominates other directions (6 of the 12 embryos). These flows have a maximum speed around 2.5–4  $\mu\text{m}/\text{min}$ . Unlike the wild-type profile, whose point of maximum flow and stall point are roughly constant in time, the *arx-2* (RNAi) flow profiles show no stall point, are larger in magnitude, and have a local maximum which is positioned far to the anterior of the wild-type.

We hypothesized that the chaotic and noisy flow speeds in *arx-2* (RNAi) embryos were a result of the smaller anterior cap being pulled and pushed around by forces from the mitotic spindle. Because of this, we applied nocodazole, a drug which depolymerizes microtubules, and observed the resulting dynamics. Interestingly, nocodazole treatment of wild type embryos appears to remove the posterior-directed flow earlier in early maintenance phase (Fig. ??) (Sailer et al., 2015). In late maintenance, however, the dynamics are relatively unchanged with nocodazole treatment, with a peak in the myosin concentration around 40% embryo length and a maximum flow speed of 2  $\mu\text{m}/\text{min}$  at 60% embryo length (blue lines in Fig. ??).

In *arx-2* (RNAi) embryos, nocodazole treatment significantly improved the patterns of flow, confining them to the A/P axis and allowing us to extract meaningful information. Similar to the case without nocodazole, embryos treated with *arx-2* (RNAi) and nocodazole showed a hypercontractile anterior myosin cap (Fig. ??), with the cap continuing to contract over time to the very edge of the anterior domain. The contraction of the cap corresponded with marked increases in flow speed during maintenance, so that flows went from 1.5  $\mu\text{m}/\text{min}$  during the third minute before anaphase onset to 3–4  $\mu\text{m}/\text{min}$ , with the point of maximum flow occurring at 40% embryo length instead of 60% (Fig. ??). Thus, regardless of the presence of microtubules, depletion of *arx-2* by RNAi leads to a hypercontractile state with a larger velocity and a cap which tends to fall off the edge of the embryo, similar to what we observed in simulations in Fig. ???. We now propose a model for how branched actin could inhibit contractility and lead to the formation of a stable boundary.

## **Incorporating branched actin into existing models correctly reproduces rescue dynamics**

To model branched actin, we work off the following two important observations from our maintenance-phase rescue experiments: first, the pinning of the myosin domain and drop in velocity happen quite abruptly, and second, the rapid drop in velocity is *not* accompanied by strong changes in the myosin concentration profile (Fig. ??). These observations together suggest that there is an ultra-sensitive dependence of contractility (not myosin concentration) on branched actin. As rescue progresses, branched actin becomes active on the anterior, providing a “brake” which slows down contractility

and progression of the boundary.

To translate our hypothesis into our model, we make the following assumptions,

1. To activate branched actin, we assume a threshold of CDC-42 above which branched actin is created. The threshold is reached roughly when the A/P boundary reaches the middle of the cell.
2. Branched actin assembly is autocatalytic, since new branches become sites for additional branches.
3. Branched actin unbinds from the cortex with characteristic rate  $k_R^{\text{off}}$  (about 8 s lifetime).
4. While branched actin does not diffuse in the cortex, polymerization of actin leads to a spreading out of the branched network, and so we include nonzero diffusivity.
5. Branched actin inhibits contractility.

The first four assumptions lead to the advection-diffusion-reaction equation for branched actin given in the SI. For the fifth assumption, we postulate a simple relationship for how branched actin impacts contractile stress, given in the SI.

The dynamics of rescue in this case are shown in Fig. ???. In the early stages of rescue, we see a concentration of myosin and CDC-42 into an interior peak which grows over time. Once the concentration of CDC-42 at the peak exceeds the threshold  $C_R = 0.25$ , branched actin is produced. This causes an immediate drop in contractility within the peak (see the active stress profile at intermediate times), which leads to a leveling out of the anterior profiles of CDC-42 and myosin (weaker flows in the anterior allow CDC-42 to diffuse over). Following this, branched actin builds up on the anterior. The boundary stops moving when the tension on the anterior balances the tension on the posterior, leaving an intermediate zone of contractility at around 60% embryo length.

A necessary feature of our simulations is the threshold dependence of branched actin production on CDC-42 activity.

## Discussion

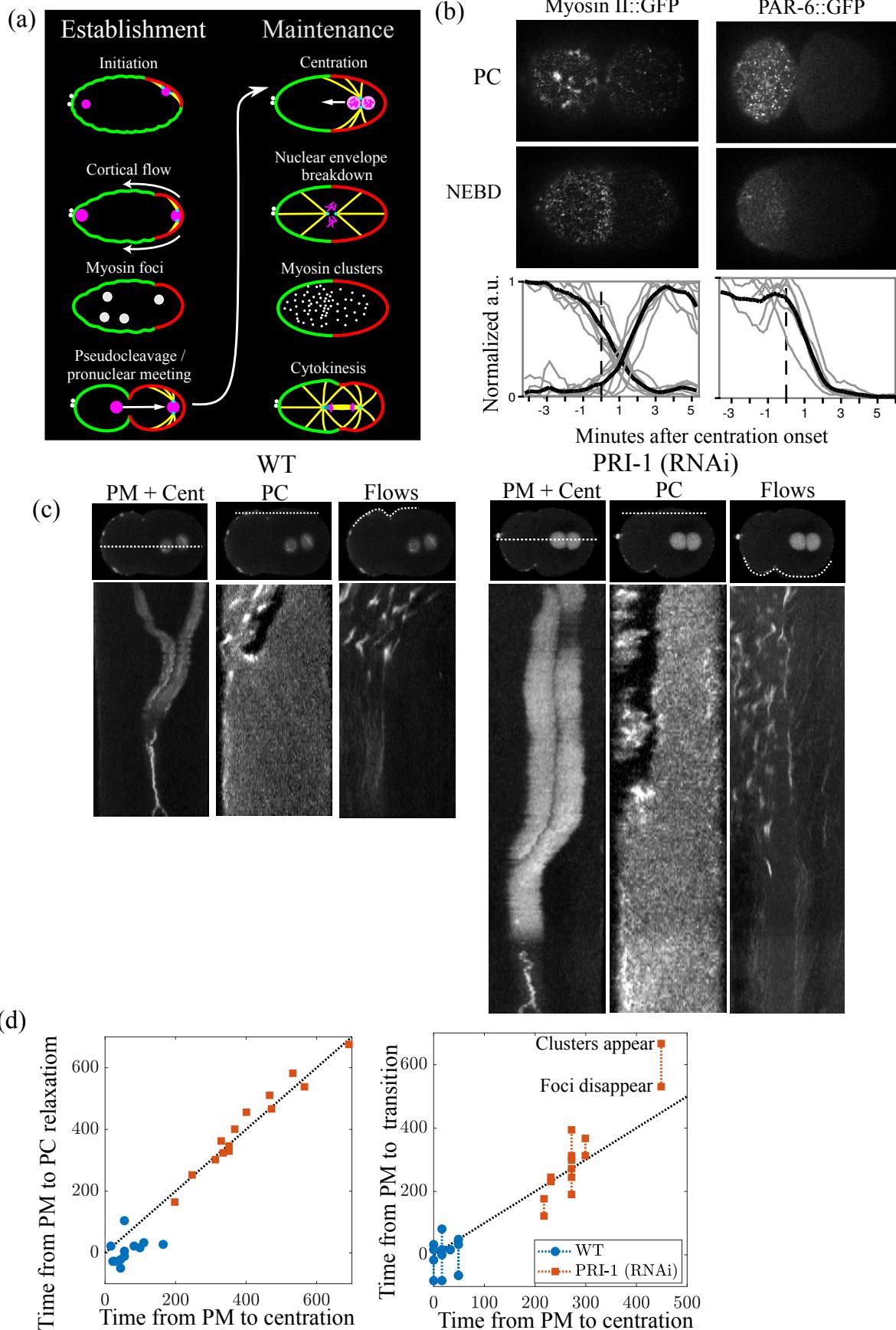


Figure 1: Cell cycle progression controls a regime change from rho-dependent to CDC-42-dependent contractility. (a) Overview of dynamics during establishment and maintenance phase, including key events in the cell cycle. (b) Distributions of non-muscle-myosin II (NMY-2::GFP; left panels) GFP::PAR-6 (right panels) in wild-type embryos at/near the end of pseudocleavage (top panels) or nuclear envelope breakdown (bottom panels). Graphs show the normalized intensity of myosin foci and clusters (left), or PAR-6 clusters (right) over time relative to centration onset. (c) Kymographs showing the correlation between pronuclear meeting and centration (left), pseudocleavage relaxation (middle), and flow into the pseudocleavage (right) in wild type (left) and PRI-1 (RNAi) embryos. (d) Correlation between centration and pseudocleavage relaxation (left) and centration and myosin transitions (right) in wild-type (blue circles) and PRI-1 (RNAi) embryos (red squares). **First draft complete.**

Figure 2: Maintenance phase biochemistry encodes a stable, attractive steady state with a unique boundary position. (a) CDK-1, (b) ect-2 ts, (c) Charlie’s data.

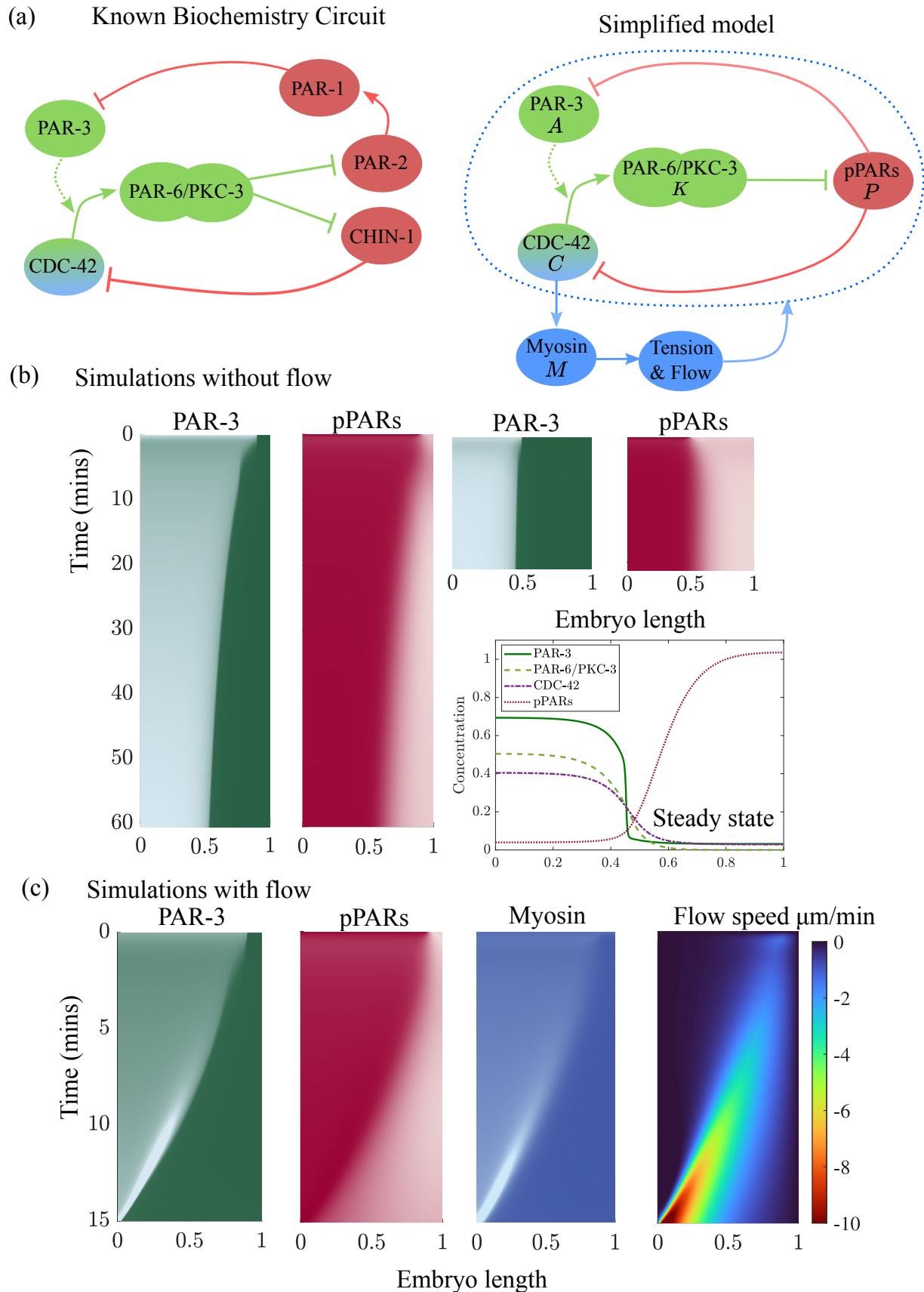
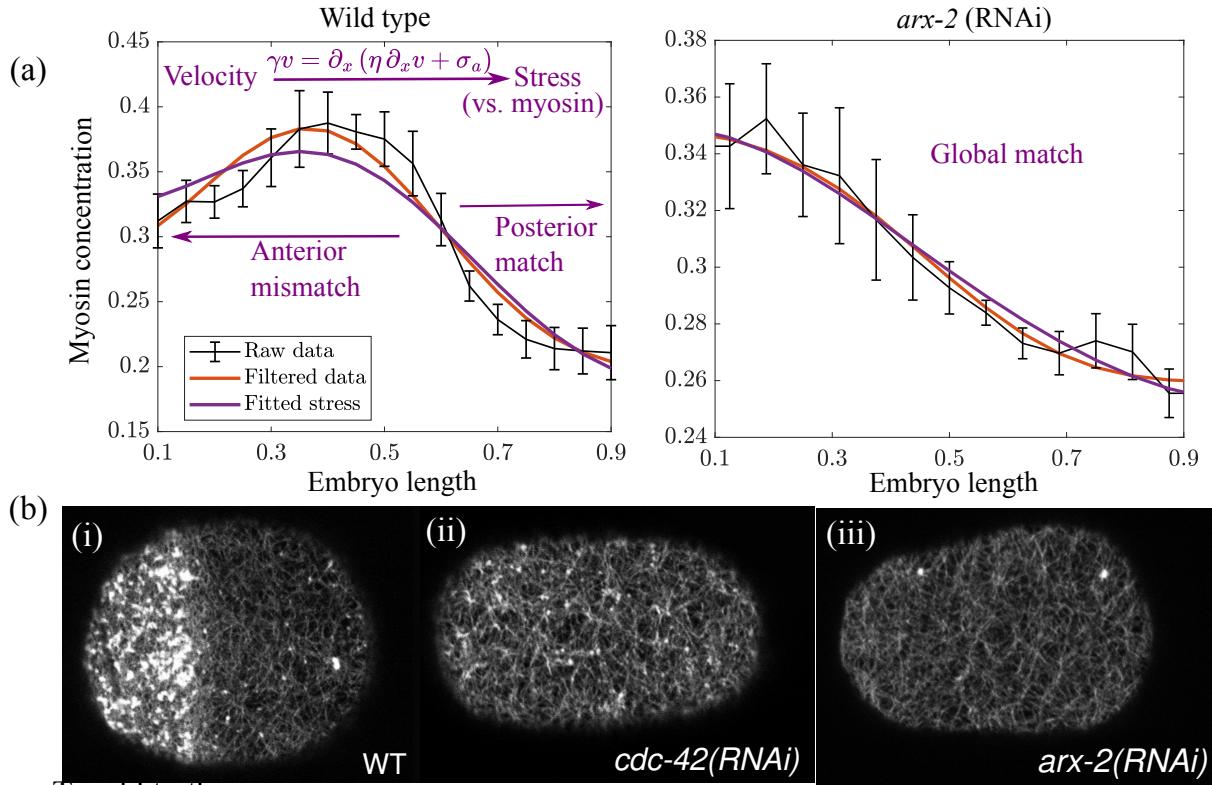


Figure 3: Models based a simple interaction of PAR proteins and myosin can stably maintain a polarity boundary, but cannot explain rescue. (a) Simplified model of PAR protein dynamics, and how it relates to the known system biochemistry (Lang and Munro, 2017). (b) Simulations without flow can maintain a stable boundary but cannot reproduce rescue in a realistic time. These panels show pseudo-kymographs of PAR-3 (green, left) and pPARs (red, right) in rescue (left, the boundary starts at 90% embryo length) and normal maintenance (right, the boundary starts at 50% embryo length) conditions. The bottom plot shows the unique steady state in the model without flow. (c) Simulations with flow lead to a vanishing anterior domain. Shown are pseudo-kymographs of the PAR-3, pPAR, and myosin concentrations over time, as well as the flow speeds. **First draft complete**



To add to this:

Branched actin phenotype with and without nocodazole  
Pictures and kymographs

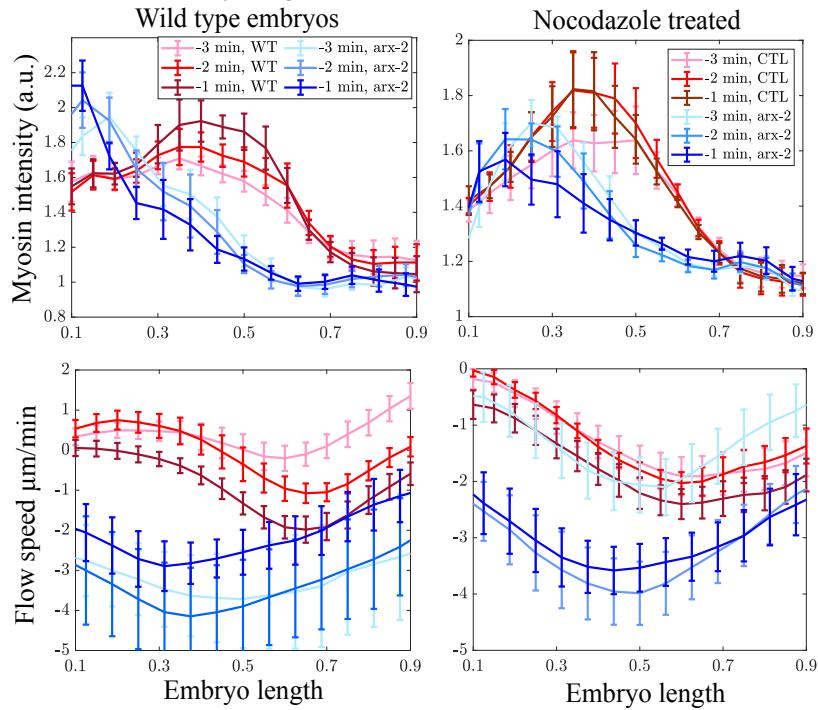


Figure 4: Branched actin acts in the anterior to suppress contractility in wild-type embryos.

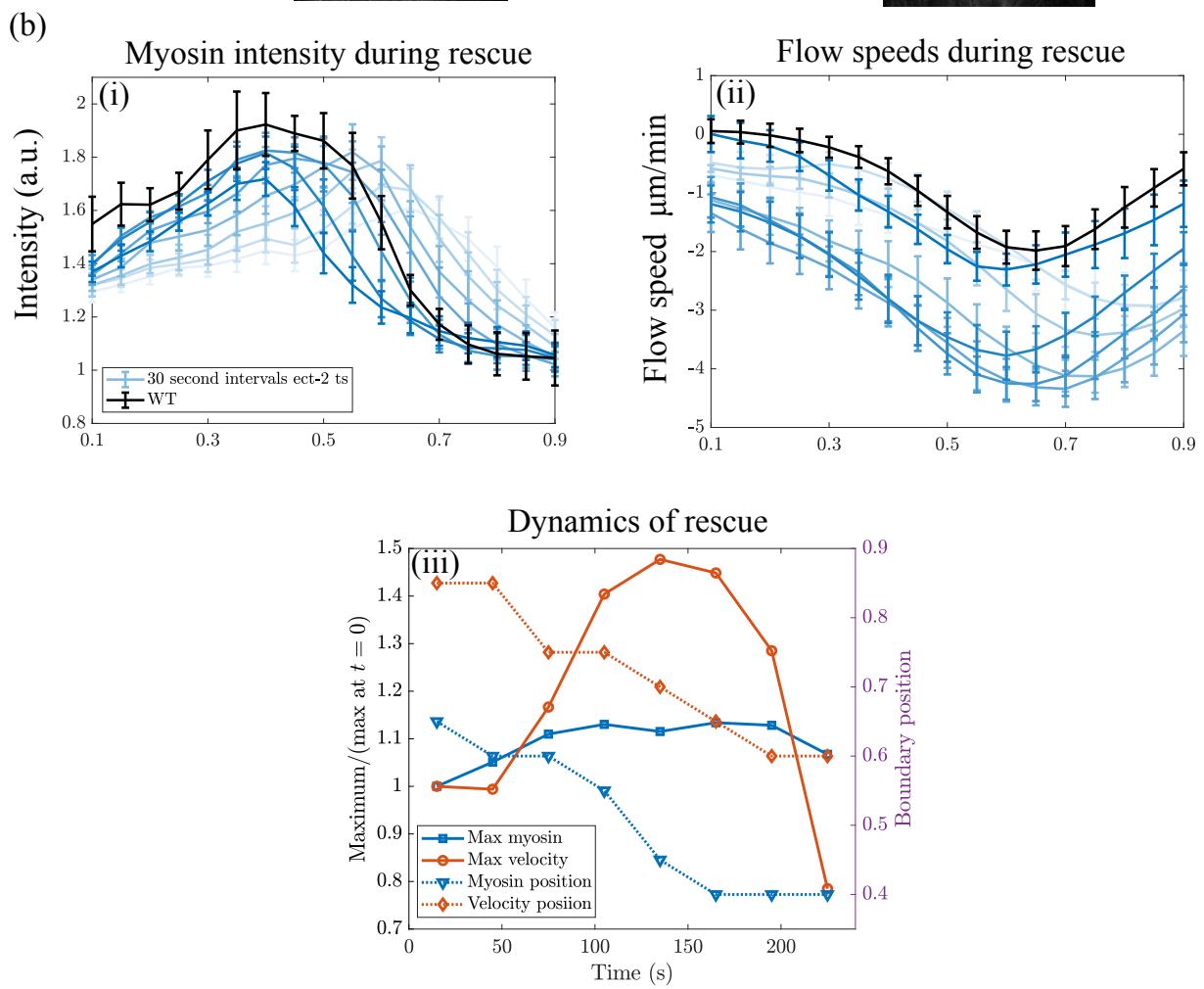
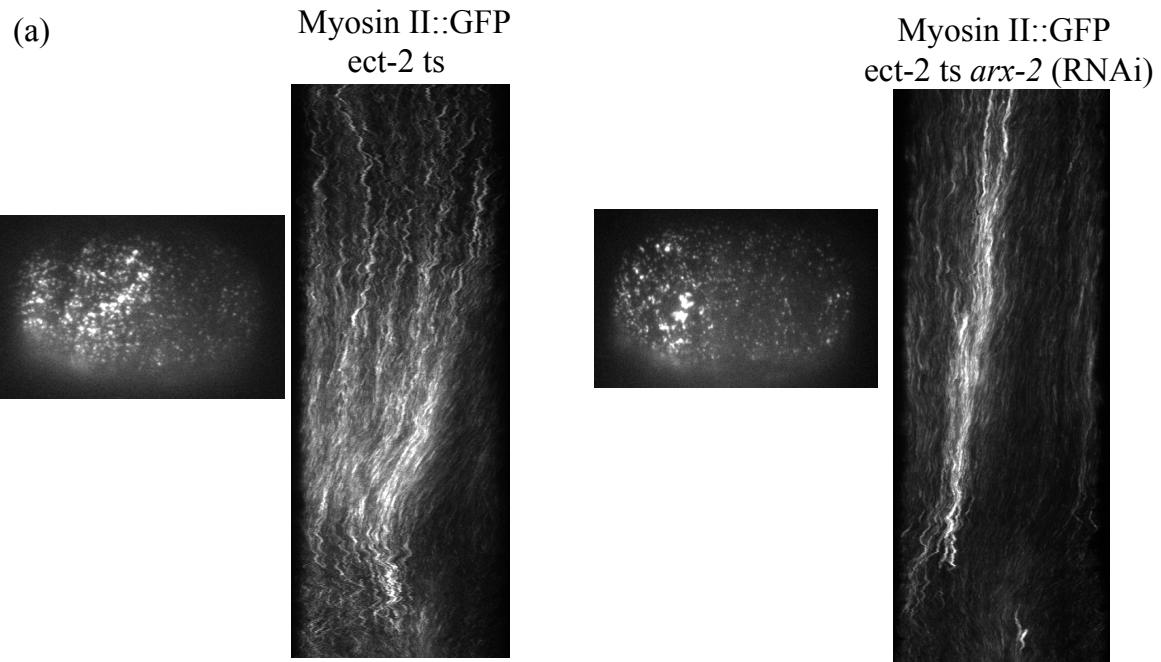
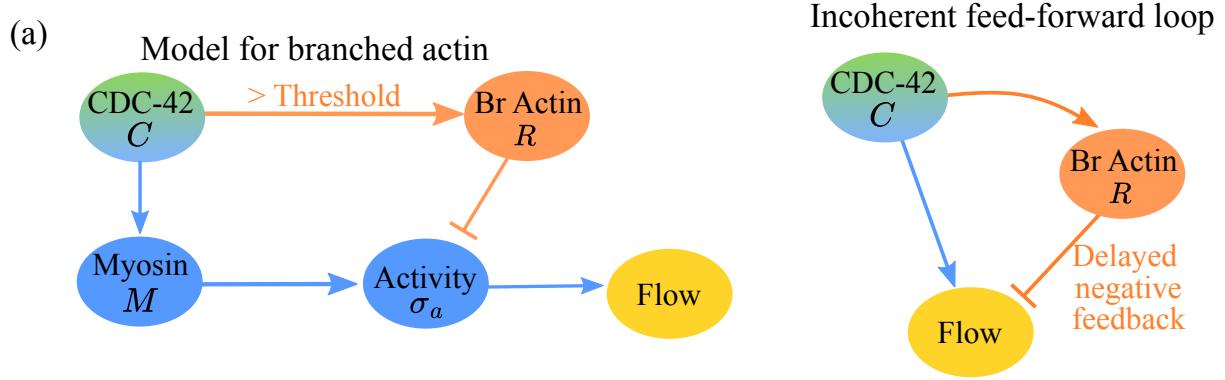


Figure 5: Branched actin acts suddenly to alleviate contractile tension in the anterior during maintenance phase rescue. (a) Still images and kymograph of myosin II (NMY-2::GFP) in ect-2 ts embryos (left) and ect-2 ts embryos with arx-2 (RNAi) (right). (b) Myosin intensity (i) and flow speeds (ii) in ect-2 (ts) embryos (blue lines) compared to wild-type. Data are shown at 30 second intervals using progressively darker lines, and wild-type data in the last minute of maintenance phase are shown in black. (c) Summary of rescue dynamics. Solid lines, governed by the left axis, show the maximum in myosin intensity (blue) and velocity (red), scaled by the value at the onset of maintenance phase ( $t = 0$ ). Dashed lines, governed by the right axis, show the position of the peak in myosin (blue) and velocity (red) over time. **First draft complete.**



(b)

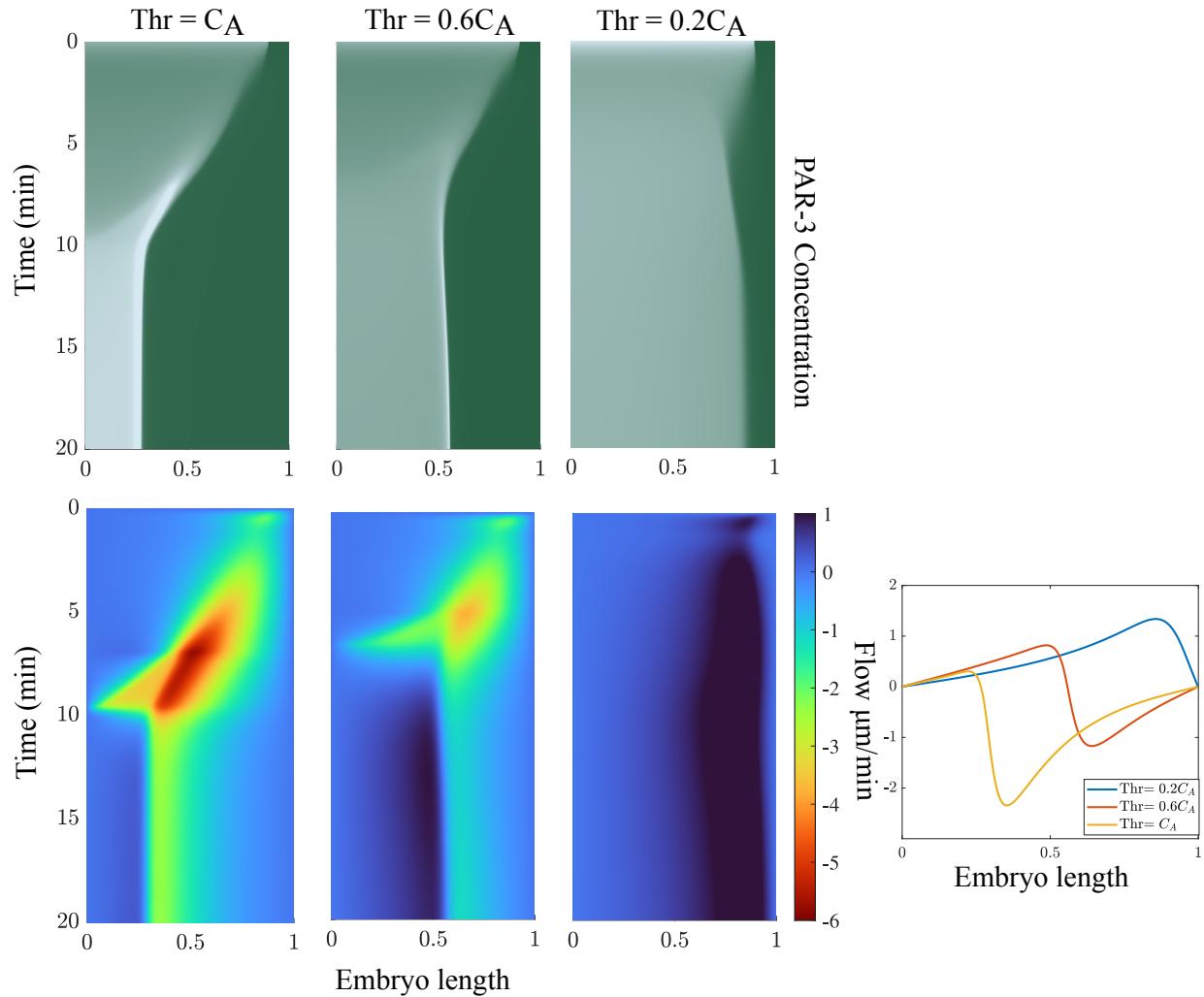


Figure 6: Models that incorporate branched actin successfully reproduce the dynamics of rescue. (a) Extension of the model presented in Fig. 3(a) to account for branched actin. Above a threshold of CDC-42, branched actin is produced and inhibits contractile stress. At its simplest level, these dynamics are an incoherent feed-forward loop (right). (b) Simulations with varying branched actin thresholds show varying degrees of contractility. Shown are kymographs of the PAR-3 domain (top) and flow speeds (bottom) over time. Each column shows a different threshold for branched actin, given in terms of the typical anterior CDC-42 concentration (0.4; see Fig. 3(b)). The steady state flow profiles for each threshold are also shown at bottom right. **First draft complete.**

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