PAR protein activation-deactivation cycles stabilize long-axis polarization in *Caenorhabditis elegans*

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Cell polarity endows cells with a reference frame that guides cellular organization and division. In the *Caenorhabditis elegans* zygote, PAR protein patterns determine the anterior-posterior axis and facilitate the redistribution of proteins for the first asymmetric cell division. While previous theoretical work has shown that mutual antagonism between (anterior) aPAR and (posterior) pPAR proteins is the key to polarity maintenance, what factors determine the selection of the polarity axis remains unclear. Here we formulate a reaction-diffusion model in a realistic cell geometry, based on bimolecular reactions and fully accounting for the coupling between membrane and cytosolic dynamics. We find that the kinetics of the phosphorylation-dephosphorylation cycle of PAR proteins is crucial for the selection of the long (anterior-posterior) axis for polarization. Biochemical cycles based on mutual exclusion alone, without a delay in dephosphorylation, would lead to short (dorsal-ventral) axis polarization. Our analysis shows that the local ratio of membrane surface to cytosolic bulk volume is the main geometric cue to which patterns adapt, and the decisive parameter that determines axis selection is given by the ratio of the diffusive length of the phosphorylated (inactive) phase to the cell length. We quantify the effect of relative protein numbers and find that they primarily affect the robustness of protein pattern formation. In particular, robustness to variations in the phosphorylation rates increases if scaffold proteins like PAR-3 are more abundant than PKC-3, which phosphorylates pPARs. Together, our theoretical study reveals the crucial role of geometry in self-organized protein pattern formation: axis selection is based on the generic dependence of intracellular pattern-forming processes on the local ratio of membrane surface to cytosolic volume.

Cell polarity is a crucial process in development [1]. Well studied examples include localization of bud sites in *Saccharomyces cerevisiae* [2], apico-basal asymmetry in mammalian epithelial cells [3], and the asymmetric placement of the first cell division in *Caenorhabditis elegans* zygote [4]. A key question in such systems is how the correct polarity axis is established and robustly maintained.

In *C. elegans*, the anterior-posterior axis of the embryo is determined in the fertilized egg by a polarized distribution of PAR (partitioning defective) proteins [4–6]. Immediately before the establishment of polarization begins, the future anterior PARs (aPARs) cover the cell cortex uniformly, while posterior PARs (pPARs) are cytoplasmic [7]. After fertilization, contraction of the actomyosin network leads to cortical flows that displace cortical aPARs anteriorly, allowing cytoplasmic pPARs to bind to the posterior zone [8, 9]: see Fig. 1A [10]. Once these two PAR domains have formed (during the ‘establishment phase’) and have thereby established the anterior-posterior axis, they persist for several minutes through the ‘maintenance’ phase until cell division [5, 7].

Several independent *in vivo* experiments on *C. elegans* have demonstrated that maintenance of PAR protein polarity is independent of an intact actomyosin network [7, 11–14]. Rather, it appears that the entry of the sperm and the following contractions of the cortical actomyosin serve as a temporal trigger for the establishment of the PAR protein pattern [9, 12, 15]. How then is formation of the pattern biochemically established and how is it stably maintained? Based on the fact that aPAR and pPAR proteins mutually drive each other off the membrane by phosphorylation [16], and that this antagonism promotes formation of distinct domains on the membrane [17–19], previous studies have outlined how self-organization of PAR proteins maintain polarization until cell division [14, 15]. These studies showed that basic features of PAR protein polarization can be explained by minimal reaction-diffusion models. However, as these models used a simplified one-dimensional geometry and assumed that cytosolic proteins are homogeneously distributed, the effect of cell geometry was disregarded and the distinction between long and short axis was lost. Thus, how the long axis is selected for polarization and subsequently maintained, and in a broader context, which features of a reaction-diffusion system are responsible for axis selection remain open questions.

To answer these questions we draw on previous studies of other intracellular pattern-forming protein systems which revealed that even the typically rather fast cytosolic diffusion does not eliminate protein gradients in the cytosol [20–23]. As a consequence, protein patterns are generically sensitive to cell geometry through coupling between processes in the cytosol and on the membrane. In particular, it was predicted [20, 21] that delayed reattachment to the cell membrane (e.g., due to cytosolic nucleotide exchange) is key to geometry sensing. Indeed,
recent experimental studies support the idea that axis selection depends on the interplay between reaction kinetics and cellular geometry [22].

These results suggest that the protein dynamics in the cytoplasm of the C. elegans embryo may also influence the selection of the long over the short axis during polarity maintenance. In order to investigate axis alignment, we developed a reaction-diffusion model of the PAR protein dynamics in the fertilized egg. As in previous studies [9, 14, 24], a central element in our model is mutual displacement of membrane-bound aPARs and pPARs by phosphorylation. However, in contrast to earlier models [9, 25], we do not use effective nonlinearities but strictly biomolecular reactions based on mass-action law kinetics, e.g. by explicitly modeling the formation of PAR protein complexes. Importantly, we also account for the delay caused by the need for reactivation of detached PAR proteins by cytosolic dephosphorylation, thus introducing the generic feature of a biochemical activation-deactivation cycle. As we will show, this feature is of particular relevance in a realistic cell geometry where diffusion and reactions of proteins on the membrane and in the cytosol are fully accounted for.

Our extended reaction-diffusion model in realistic cell geometry reveals that the dynamics of the phosphorylation-dephosphorylation cycle of PAR proteins is crucial for long-axis polarization. Without this additional feature, the biochemical network of PAR proteins would lead to the selection of short- instead of long-axis polarity in the single-cell embryo. Furthermore, we characterize the roles of mutual antagonism (phosphorylation) and overall protein numbers in robust long-axis polarization: while the phosphorylation rates determine how distinctively one polarization axis is selected over the other, relative protein numbers primarily affect the robustness of pattern formation as a whole.

Most importantly, our analysis indicates that these findings can be generalized beyond the specific model for the PAR system: axis selection is based on the generic dependence of intracellular pattern forming processes on the local ratio of membrane surface to cytosolic volume. Broadly speaking, this ratio determines the likelihood that a given protein will reattach to the membrane after detachment into the cytosol.

**REACTION-DIFFUSION MODEL**

The aPAR set of proteins comprises PAR-3, PAR-6, and the atypical protein kinase PKC-3. Only complexes containing PKC-3 can phosphorylate pPARs, thereby disabling their membrane-binding capacity [17, 26]. How trimeric complexes consisting of PAR-3, PAR-6 and PKC-3 actually form is not fully understood. The evidence so far suggests that PAR-6 acts as a linker between PKC-3 and PAR-3, which can itself bind directly to the membrane [27–30]. In the absence of PAR-6, PKC-3 freely diffuses in the cytosol [31, 32]. In the reaction

![Diagram](image)

**FIG. 1.** (A) Cell polarization in the C. elegans embryo during the establishment (top) and maintenance (bottom) phases; sketch adapted from Ref. [5]. (B) Illustration of the protein flux between cytosol and membrane. As proteins detach from the membrane when phosphorylated, they cannot immediately rebind to the membrane. There is therefore an intrinsic delay before dephosphorylation permits rebinding. (C) The biochemical reaction network is comprised of two mutually antagonistic sets of proteins, aPARs and pPARs. Dephosphorylated (active) A1 and P attach to the membrane with rates $k_{a1}$ and $k_{p1}$, respectively. Both active proteins may also detach spontaneously from the membrane with rates $k_{a2}^{off}$ and $k_{p2}^{off}$, respectively. A1 acts as a scaffold protein: Once bound to the membrane it recruits A2 with rate $k_{A2}$ and forms a membrane-bound heterodimeric aPAR complex A12. The heterodimer A12 may itself spontaneously detach from the membrane with rate $k_{A2}^{off}$ and dissociate into A2 and active A1. Membrane-bound A1 and A12 can also be phosphorylated by P with rate $k_{P1}^{off}[P]$, thereby initiating dissociation of the aPAR complex and release of the aPAR proteins into the cytosol. While reattachment of the scaffold protein A1 is delayed by the requirement for dephosphorylation (reactivation), detached A2 can be recruited to the membrane by membrane-bound A1 immediately. Similarly, P is phosphorylated by the heterodimer A12 at rate $k_{P1}[A12]$, and is consequently released as inactive P into the cytosol. In the same way as A1, also P must be dephosphorylated before it can bind again to the membrane. For simplicity, we take identical dephosphorylation (reactivation) rates $\lambda$ for inactive A1 and P. The ensuing reaction-diffusion equations and a table listing the values of the rate constants are provided in the SI section S.I..
Cytosolic dephosphorylation plays a key role for axis determination

For mutually antagonistic protein interactions as in the PAR system, protein domains are separated by an interface at which mutually induced membrane detachment dominates. It is assumed to be recruited by scaffold proteins $A_1$ that are already bound to the membrane, thereby forming hetero-dimers $A_{12}$ on the membrane which correspond to trimeric complexes. These complexes can then phosphorylate membrane-bound pPARs, which initiates their release into the cytosol in a phosphorylated (inactive) state.

As with aPARs, there are different pPAR species, PAR-1 and PAR-2. While it is known that PAR-2 binds directly to the membrane, and PAR-1 phosphorylates PAR-3, it remains unclear whether PAR-2 also helps to maintain anterior-posterior polarity by excluding aPAR complexes from the membrane. However, PAR-2 is required for posterior binding of PAR-1 and PAR-2 exclusion from the membrane by PKC-3 is essential for proper restriction of pPARs to the posterior. In view of the remaining uncertainties, and for the sake of simplicity, we refrain from distinguishing between different species and effectively treat the pPARs as a single species $P$ (Fig. 1C). $P$ phosphorylates membrane-bound $A_1$ and $A_{12}$, which triggers their subsequent detachment as a phosphorylated (inactive) species into the cytosol.

To provide a realistic cell geometry (prolate spheroid) similar to that previously employed in studies of Min oscillations in E. coli, we use a two-dimensional elliptical geometry with long axis $a \approx 27 \mu m$ and short axis $b \approx 15 \mu m$. The boundary and interior of the ellipse represent the cell membrane and cytosolic volume, respectively. In addition to cytosolic diffusion, our model also accounts for protein dephosphorylation reactions in the cytosol. This creates deactivation-reactivation cycles, as proteins that were phosphorylated (deactivated) on the membrane are thereby reactivated for membrane binding (Fig. 1B, C). For simplicity, the reactivation (dephosphorylation) rate $\lambda$ is assumed to be identical for cytosolic pPARs ($P$) and aPARs ($A_1$). The ensuing reaction-diffusion equations are given in section S.I. of the Supplementary Material.

RESULTS

Cytosolic dephosphorylation plays a key role for axis determination

For mutually antagonistic protein interactions as in the PAR system, protein domains are separated by an interface at which mutually induced membrane detachment dominates. For this interface (and the protein domains it separates) to be maintained, proteins that have detached from the membrane must be replaced, otherwise the antagonistic interaction between the proteins would deplete either aPARs or pPARs from the membrane. As the protein interactions are mass-conserving, maintenance requires that detached proteins quickly rebinding, unless the cytosolic reservoir of proteins is large enough for them to be replenished directly. This suggests that an interface can best be maintained in those membrane regions where rebinding to the membrane after detachment is most likely.

The likelihood of rebinding is in turn determined by the availability of cytosolic proteins for binding, which is a function of the interplay of two factors: the local cell geometry and the time required for reactivation of the detached proteins by dephosphorylation (Fig. 2). Due to different local membrane curvatures in a cell, the ratio of available membrane surface to cytosolic volume is highest at cell poles and lowest at midcell. How the local cell geometry affects protein rebinding depends on the dephosphorylation time: a longer reactivation time implies that a protein that detached in a phosphorylated state from the membrane will on average diffuse farther away from the membrane before it can be reactivated and reattaches. As in a standard diffusion-degradation problem, the corresponding reactivation length is estimated at cell poles and lowest at midcell.
timated as $\ell := \sqrt{D_{\text{cyt}}/\lambda}$.

To see how this diffusion length affects protein dynamics, consider a protein with a short inactive (phosphorylated) phase, such that $\ell$ is significantly smaller than the cell size $L = 2a$ (Fig. 2A). Then, proteins are likely to be dephosphorylated fast and can therefore rebind very soon after phosphorylation-induced detachment. Since the local ratio of membrane surface to cytosolic volume at the cell poles is larger than at midcell, these proteins are more likely to reencounter the membrane in the polar zone which translates into higher polar reattachment (after reactivation), i.e. proteins remain caged at the cell poles (Fig. 2A). Conversely, proteins that detached from the membrane at midcell have more cytosolic volume available than those that detached at the poles and, thus, are less likely to re-encounter the membrane and rebind there (Fig. 2A). This heuristic picture suggests that for $\ell \ll L$ domain interfaces preferentially form at the cell poles and hence cell polarity will be established along the short axis.

If dephosphorylation requires more time, $\ell$ increases and the effect of local membrane curvature (ratio of membrane surface to cytosolic volume) is attenuated (Fig. 2B). As the reactivation length approaches the cell size ($\ell \lesssim L$), the farther detached proteins can diffuse from their detachment position. Ultimately, when $\ell > L$, proteins can be considered as uniformly distributed throughout the cytosol for the next attachment event (Fig. 2D). Therefore, reactivated proteins are more likely to attach at midcell, where the accumulated density along the long axis (or, equivalently, the ratio of cytosolic volume to membrane area) is highest (Fig. 2C). This implies that an interface between different protein domains will establish itself at midcell and cells will become polarized along the long axis for large enough reactivation length $\ell$.

In summary, if cell polarization is induced by antagonistic protein interaction as in *C. elegans*, we expect long axis polarization only if the delay resulting from the inactive phase is sufficiently long. Moreover, our heuristic analysis also suggests that relative protein numbers affect axis selection, as the global availability of an abundant protein species attenuates effect of cell geometry associated with the activation-deactivation cycle.

### Linear stability analysis

To put the above heuristic reasoning on a firm basis and gain a deeper understanding of the mechanisms underlying axis selection, we performed a mathematical analysis, building on previous investigations of intracellular pattern formation in elliptical cell geometry [20, 21].

Importantly, in the bounded geometry of a cell, broken detailed balance due to the dephosphorylation-phosphorylation cycle – more generally an activation-deactivation cycle – implies that a uniform well-mixed state can no longer be a steady state of the system [21].

Instead, all steady states show cytosolic gradients with a density profile that is spatially non-uniform but unpolarized [21]. These near-uniform steady states can be well approximated by states with a homogeneous protein concentrations on the membrane and a cytosolic protein gradient perpendicular to the membrane [20]; see also section S.II. in the Supplementary Information. As the reactive dynamics in the PAR system is bistable, there are two such unpolarized states, one with aPAR and the other with pPAR being the more abundant membrane species. In the zygote, aPARs predominate on the membrane, and we refer to this aPAR-dominant state as the unpolarized state.

To perform a linear stability analysis with respect to this unpolarized state, we use Fourier modes specific for the elliptical cell geometry [20]. These modes are classified as even and odd by their symmetry with respect to reflections through a plane along the long axis, and correspond to membrane patterns aligned along the long and short axes, respectively (Fig. 3A). If the real parts of the growth rates $\sigma$ of all Fourier modes are negative, small spatial perturbations of the unpolarized state will decay and it will remain stable. In contrast, a positive real part of any growth rate indicates that the unpolarized
state is unstable to spatial perturbations, and initially
a pattern will emerge corresponding to the mode with
the highest growth rate (Fig. 3B). Hence, linear stability
analysis informs about the parameter regime where pat-
terns of a certain symmetry (short vs. long axis) form
spontaneously. We expect that these pattern attractors
persist for some range outside the linear unstable param-
eter regime (see section S.III. in the SI), where patterns
do not form spontaneously but can be triggered by a fi-
nite perturbation — such as the fertilization event.

For a typical cell size and cytosolic diffusion constants
in the range of 5–50 \( \mu \text{m}^2/\text{s} \), our linear stability analysis
shows that second- and higher-order modes are negligi-
ble compared to the first even and odd modes, \( \sigma_e \) and \( \sigma_o \),
respectively. In the parameter regime under considera-
tion, those two growth rates exhibit similar magnitude
and at least one of them is positive. To quantify the
competition between the first even and odd modes (long
vs. short axis), we define the relative difference in their
growth rates, \( \delta \sigma := (\sigma_e - \sigma_o)/\sqrt{\sigma_e^2 + \sigma_o^2} \); for an illustration
see Fig. 3B.

Cytosolic reactivation length determines the
polarization axis

We computed \( \delta \sigma \) as a function of \( \lambda \) and \( D_{\text{c}} \). As
shown in Fig. 3C, the even mode dominates (\( \delta \sigma > 0 \)) for
\( \ell > 15 \mu \text{m} \); otherwise the odd mode dominates. This is
consistent with the above heuristic reasoning suggesting
that reactivation must be slow or cytosolic diffusion must
be fast for long-axis polarity. To test whether the lin-
ear stability analysis correctly predicts the final polar-
ization axis, we performed finite-element (FEM) simu-
lations; see also section S.III. in the SI. These simulations
show that there is a threshold value for the reac-
tivation length \( \ell^* = 11.2 \mu \text{m} \) (approximately 20% of the
cell length \( L \)) above/below which cells stably polarize
along the long/short axis (Fig. 3C). We conclude that
the reactivation length \( \ell \), which determines the spatial
distribution of active proteins, is the decisive parameter
that determines axis selection.

Relative phosphorylation rates restrict the
spontaneous emergence of polarization

Whether there is a spatial separation between aPAR
and pPAR domains, is known to depend on the rela-
tive magnitude of the phosphorylation rates \( k_{\text{AP}} \) and
\( k_{\text{pAP}} \) [9, 15]: an interface between different domains exists
and can be maintained only if these antagonistic phos-
phorylation processes are balanced. To determine the
necessary conditions for this balance, we analyzed the
stability of the unpolarized state numerically, while vary-
ing both phosphorylation rates over one order of magni-
tude. The cytosolic diffusion constant was kept fixed at
\( D_{\text{c}} = 30 \mu \text{m}^2/\text{s} \), and we chose two representative reacti-
vation rates, \( \lambda = 0.3 \text{s}^{-1} \) and \( \lambda = 0.05 \text{s}^{-1} \), corresponding
to reactivation lengths, \( \ell = 10 \mu \text{m} \) and \( \ell = 24.5 \mu \text{m} \), re-
spectively.

Our analysis in full cell geometry shows that sponta-
eseous polarization starting from the unpolarized state
arises only within a limited range of \( k_{\text{pAP}}/k_{\text{AP}} \) values (cones in Fig. 4), in accordance with previous theoretical
studies using a simplified one-dimensional model [9, 25].
Strikingly, our results also show that the selection of the
polarization axis does not depend on the nonlinear pro-
cesses (mutual antagonism) but primarily on the linear
activation-deactivation cycle, i.e. the relative magnitude
of reactivation length and cell size, \( \ell/L \). The ratio of the
phosphorylation rates mainly determines the initial
preference for a polarization axis starting from an unpo-
larized state (Fig. 4A, B).

Specifically, we find that for \( \lambda = 0.3 \text{s}^{-1} \), the first even
mode grows more slowly than the first odd mode (\( \delta \sigma < 0 \)),
favoring short-axis polarization. The results are more di-
verse for \( \lambda = 0.05 \text{s}^{-1} \). Here, for large \( k_{\text{pAP}}/k_{\text{AP}} \), the first
even mode grows faster than the first odd mode (\( \delta \sigma > 0 \)).
In contrast, for the mid to low range of \( k_{\text{pAP}}/k_{\text{AP}} \), one
finds \( \delta \sigma \approx 0 \), i.e. linear stability analysis does not predict
a clear preference for either long- or short-axis polar-
ization. FEM simulations show, however, that – irrespec-
tive of the ratio \( k_{\text{pAP}}/k_{\text{AP}} \) — long- and short-axis polarization
in the final steady state is obtained for \( \ell = 10 \mu \text{m} \) and
\( \ell = 24.5 \mu \text{m} \), respectively; see S.III. in the SI. These sim-
ulations confirm that the reactivation length \( \ell \) is the de-
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![FIG. 4. Role of phosphorylation rates for polarization and axis selection.](image-url)
Robustness of polarization as well as axis selection depend on the relative protein densities

After learning that the abundance of cytosolic proteins determines initial axis selection, we asked how changing the relative total protein densities affects cell polarization. A linear stability analysis revealed that density variations alter several features: the range of ratios \( k_{Pa}/k_{Ap} \) for which an interface between different PAR domains can be stably maintained, and the threshold value of reactivation length \( \ell^* \) that distinguishes between short- and long-axis polarization. The effects were most prominent when the ratio of pPAR and aPAR proteins that phosphorylate each other ([\( P \)/[A2]], and the ratio of aPAR proteins ([\( A1\)/[A2]]) was varied.

As shown in Fig. 5, varying the ratio of the antagonistic proteins ([\( P \)/[A2]]) mainly shifts the regime of spontaneous cell polarization up on the \( k_{Pa}/k_{Ap} \) axis. This shift is easily explained, as the effective mutual phosphorylation rates are given by \( k_{Ap}[P] \) and \( k_{Pa}[A12] \), respectively – where \([A12]\) is mainly limited by the availability of \([A2]\). Therefore, when the concentration of pPAR proteins ([\( P \)]) is increased relative to \([A2]\), the per capita rate \( k_{Pa} \) has to be increased relative to \( k_{Ap} \) as well, in order to retain the balance between the mutual phosphorylation processes.

Changing the ratio between the different types of aPAR proteins has two effects. First, spontaneous polarization is possible for a broader range of \( k_{Pa}/k_{Ap} \). Increasing the concentration of the scaffold protein \([A1]\) relative to \([A2]\), which phosphorylates pPARs, decreases the lower bound of \( k_{Pa}/k_{Ap} \) that allows for polarization. This is a consequence of the increased reservoir size of \( A1 \) which implies a higher rate of attachment of cytosolic \( A1 \) to the membrane and hence a fast local redimerization of \( A2 \) (which lacks an inactive phase) right after the detachment of a heterodimer \( A12 \). This newly formed heterodimer \( A12 \) is then competent to phosphorylate pPARs. Thus it is plausible that even for low \( k_{Pa}/k_{Ap} \) one can achieve a balance of mutual antagonism, extending the lower bound of the polarization regime. Second, changing the ratio \([A1]/[A2]\) also has a major effect on the threshold value of the reactivation length \( \ell^* \). We find that \( \ell^* \) increases with increasing concentration of the scaffold protein \([A1]\) (Fig. 5). Again, this can be understood as a reservoir effect: globally abundant \( A1 \) promotes immediate re-dimerization of \( A2 \) with any available \( A1 \). Axis selection is then affected by the polar recycling of \( A2 \).

Taken together, both of these findings emphasize the importance of the activation-deactivation cycle. A cell polarizes more robustly for higher amounts of scaffold proteins. However, at the same time, the cytosolic reactivation length has to increase significantly in order to also robustly maintain long-axis polarization.

**DISCUSSION**

Here, we have addressed two linked questions concerning cell polarity in *C. elegans*: Under what conditions do cells polarize, and what determines the polarization
axis?

Previous experiments supported by mathematical models in simplified cell geometry have indicated that a balance between mutual phosphorylation of aPAR and pPAR proteins is the key mechanism responsible for cell polarization [9, 14, 15, 35]. Our theoretical results in realistic cell geometry support this finding. In addition, we have shown here that the robustness of cell polarity to variations in the phosphorylation rates increases if the scaffold protein PAR-3 is more abundant than PKC-3, which phosphorylates pPARs. Hence, low scaffold abundance is incompatible with robust biological function. This agrees with experimental findings that the scaffold function of PAR-3 is at least partially supported by other proteins (e.g. Cdc-42 [30]). Our results suggest that it would be worthwhile to experimentally search for other scaffold proteins and test their functional roles in axis selection.

Most importantly, our theoretical analysis in realistic cell geometry reveals that the key feature of the biochemical network responsible for axis selection is the phosphorylation-dephosphorylation cycle of PAR-3 and PAR-2 that leads to a delayed rebinding after detachment from the membrane, and the absence of such a delay for rebinding of the complex of PKC-3 and PAR-6. The reactivation time ($\lambda^{-1}$) implies a cytosolic reactivation length $\sqrt{D_{\text{cyt}}/\lambda}$ which defines a cytosolic zone of inactive proteins close to the membrane. As a consequence, proteins with a small reactivation length remain partially caged at the cell poles after membrane detachment, while those with a large reactivation length are uncaged and thereby become uniformly distributed in the cytosol before rebinding. Similarly, proteins lacking a delay, like PKC-3, are available for rebinding immediately after detachment from the membrane and are thus strongly caged to the cell poles. Our theoretical analysis shows that long axis polarization is stable only for sufficiently large $\ell$. The threshold value for the reactivation length $\ell$ depends on the ratio of PAR-3 to PKC-3: a larger cytosolic pool of PAR-3 attenuates the effect of selecting the interface at midplane and at the same time strengthens the effect of PKC-3 to put the interface at the poles. Hence we predict that increasing the number of PAR-3 destabilizes long axis polarization in favor of short axis polarization.

On a broader perspective, these results show that selection of a characteristic wavelength for a pattern and selection of a polarity axis are distinct phenomena and are, in general, mediated by different underlying mechanisms (See S.II). We expect the following findings to be generic for mass-conserved intracellular protein systems: cells sense the cellular geometry by the local membrane to bulk ratio, an activation-deactivation cycle can act as a pattern axis switching mechanism, and cytosolic protein reservoirs alter the sensitivity to cell geometry. Identifying the biochemical steps that are most relevant for axis selection in other intracellular pattern forming systems is an important theme for future research.

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