Geometric cues stabilise long-axis polarisation of PAR protein patterns in *C. elegans*

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In the *Caenorhabditis elegans* zygote, PAR protein patterns, driven by mutual antagonism, determine the anterior-posterior axis and facilitate the redistribution of proteins for the first cell division. Yet, the factors that determine the selection of the polarity axis remain unclear. We present a reaction-diffusion model in realistic cell geometry, based on biomolecular reactions and accounting for the coupling between membrane and cytosolic dynamics. We find that the kinetics of the phosphorylation-dephosphorylation cycle of PARs and the diffusive protein fluxes from the cytosol towards the membrane are crucial for the robust selection of the anterior-posterior axis for polarisation. The local ratio of membrane surface to cytosolic volume is the main geometric cue that initiates pattern formation, while the choice of the long-axis for polarisation is largely determined by the length of the aPAR-pPAR interface, and mediated by processes that minimise the diffusive fluxes of PAR proteins between cytosol and membrane.

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Cell polarisation is a crucial process in development\(^1\). Well-studied examples include localisation 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 the *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 fertilised egg by a polarised distribution of PAR (partitioning defective) proteins\(^5–7\). Immediately before the establishment of polarisation begins, the future anterior PARs (aPARRs) cover the cell cortex uniformly, while posterior PARs (pPARs) are cytoplasmic\(^7\). After fertilisation, the sperm-donated centrosome induces contraction of the actomyosin network, which leads to cortical flows that displace cortical aPARRs anteriorly, allowing cytoplasmic pPARs to bind to the posterior zone\(^8–11\); see Fig. 1a. 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\(^12,13\).

Several independent in vivo experiments on *C. elegans* have demonstrated that maintenance of PAR protein polarity is independent of an intact actomyosin network\(^7,11–15\). Rather, it appears that the entry of the sperm and the following contractions of the cortical actomyosin serve as a temporal trigger for the rapid establishment of the PAR protein pattern\(^9,13,16\). However, experimental observations also suggest that while the rapid establishment and perfect position of anterior–posterior PAR domains are the result of an interplay between mechanical, hydrodynamical and biochemical mechanisms, polarisation is nevertheless robustly established (albeit with some delay) when various mechanical and hydrodynamical mechanisms are eliminated\(^10,11,17–19\). To disentangle and understand these distinct mechanisms, one needs to investigate the mechanism of self-organised polarisation by the biochemical PAR protein network. Based on the fact that aPAR and pPAR proteins mutually drive each other off the membrane by phosphorylation\(^20\), and that this antagonism promotes formation of distinct domains on the membrane\(^10,21,22\), previous studies have outlined how self-organisation of PAR proteins maintain polarisation until cell division\(^15,16,23\). These studies showed that basic features of PAR protein polarisation 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 polarisation 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\(^24–27\). 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\(^24,25\) 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\(^26\).

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. As in previous studies\(^9,15,28\), a central element in our model is mutual displacement of membrane-bound aPARRs and pPARRs by phosphorylation. However, in contrast to earlier models\(^9,29\), we do not use effective nonlinearities, but strictly biomolecular reactions based on mass-action law kinetics, e.g., by explicitly modelling the formation of PAR protein complexes. Importantly, we also account for the delay caused by the need for reactivation of detached PAR

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**Fig. 1 Biological background and model network.** **a** Cell polarisation in the *C. elegans* embryo during the establishment (top) and maintenance (bottom) phases; sketch adapted from ref. 5. **b** Illustration of protein flux between the 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, aPARRs and pPARRs. Dephosphorylated (active) **A** and **P** attach to the membrane with rates **k**\(^{a,0}\) and **k**\(^{p,0}\), respectively. Both active proteins may also detach spontaneously from the membrane with rates **k**\(^{o,1}\) and **k**\(^{p,1}\), respectively. **A** acts as a scaffold protein: once bound to the membrane, it recruits **A** by phosphorylation\(^20\), and forms a membrane-bound hetero-dimeric aPAR complex **A**\(_2\). The heterodimer **A**\(_2\) may itself spontaneously detach from the membrane with rate **k**\(^{d,2}\) and dissociate into **A** and active **A**. Membrane-bound **A** and **A** can also be phosphorylated by **P** with rate **k**\(^{P,2}\), thereby initiating dissociation of the aPAR complex and release of aPAR proteins into the cytosol. While reattachment of the scaffold protein **A** is delayed by the requirement for dephosphorylation (reactivation), detached **A** can be recruited to the membrane by membrane-bound **A** immediately. Similarly, **P** is phosphorylated by the heterodimer **A**\(_2\) at rate **k**\(^{P,2}\), and is consequently released as inactive **P** into the cytosol. In the same way as **A**, also **P** must be dephosphorylated before it can bind again to the membrane. For simplicity, we take identical dephosphorylation (reactivation) rates **λ** for inactive **A** and **P**. The ensuing reaction–diffusion equations are provided in the Methods section Model, and a table listing the values of the rate constants can be found in ref.1. **d** Sketch of the cell’s geometry: prolate spheroid with long axis **a** and short axis **b**, and with short- (left) and long-axis (right) polarisation.
proteins by cytosolic dephosphorylation, thus introducing the
generic feature of a biochemical activation–deactivation cycle.

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 polarisation. Without
this additional feature, the biochemical network of PAR proteins
would not lead to robust polarisation along the long axis, but instead
exhibit a strong tendency to first polarise along the short axis, and
polarisation would not re-align within a time that corresponds to a
typical time before cell division. Furthermore, the extended model
enables us to characterise the roles of mutual antagonism (phos-
phorylation) and overall protein numbers in robust long-axis polarisation: while the phosphorylation rates determine how dis-
tinctively one polarisation 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
generalised 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 and on the cell geometry via the length of the
interface between the two different protein domains. Broadly
speaking, the membrane-to-bulk ratio determines the likelihood
that a given protein will reattach to the membrane quickly after
detachment into the cytosol and the interface length affects both
the establishment and maintenance of long-axis polarisation.

Results

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.21,30 How trimeric complexes con-
sisting 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 membrane31–34. In the absence of PAR-6, PKC-3 freely
diffuses in the cytosol.35,36 In the reaction network upon which
our mathematical model is based, we simplify the formation of
trimeric complexes to the formation of a complex consisting of
two effective species of aPARs: A1 and A2 (Fig. 1c). The first
species, A1, models the membrane-binding function of PAR-3,
thus we also refer to it as a scaffold protein. The second species,
A2, corresponds to a complex of PAR-6 and PKC-3. It is assumed
to be recruited by scaffold proteins A1 that are already bound to
the membrane, thereby forming hetero-dimers A12 on the
membrane. 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.7–20 However,
PAR-2 is required for posterior binding of PAR-137,38 and PAR-2
exclusion from the membrane by PKC-3 is essential for proper
restriction of pPARs to the posterior21. In view of the remaining
uncertainties, we refrain from distinguishing between different
species and effectively treat the pPARs as a single species
(Fig. 1c). P phosphorylates membrane-bound A1 and A12, which
triggers their subsequent detachment as a phosphorylated
(inactive) species into the cytosol.

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 λ is assumed to
be identical for cytosolic pPARs (P) and aPARs (only A1). The
ensuing reaction–diffusion equations are given in the Methods
section Eqs. (7)–(18).

We approximate the natural shape of a C. elegans embryo by a
prolate spheroid with semi-axis lengths a = 27 μm and b = 15 μm
(see Fig. 1d). Here, a is the distance from centre to pole through a
focus along the symmetry axis, also called the semi-major axis,
while b is the equatorial radius of the spheroid, which is called the
semi-minor axis. The boundary and interior of the ellipse
represent the cell membrane and cytosolic volume, respectively.

Dephosphorylation plays a key role for axis determination. For
mutually antagonistic protein interactions, protein domains are
separated by an interface at which mutually induced membrane
detachment dominates.3,15,16 For its maintenance, 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 rebind, unless the cytosolic reservoir of
proteins is large enough for them to be replenished directly. This
suggests that an interface can best be maintained locally in those
membrane regions where rebinding to the membrane after
detachment is most likely.

The likelihood of re-binding depends on the availability of
cytosolic proteins for binding, which depends on the interplay
between the local cell geometry and the time required for reactiva-
tion of detached proteins by dephosphorylation (Fig. 2). The ratio
of available membrane surface to cytosolic volume is highest at cell
cples and lowest at mid-cell. How this local cell geometry affects
protein re-binding 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. The corresponding reactivation length is estimated as

$$\ell := \sqrt{\frac{D_{\text{cyt}}}{\lambda}}.$$  (1)

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 length $L = 2a$
(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 mid-cell, these proteins are
more likely to re-encounter 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 mid-cell 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 is
attenuated (Fig. 2b). 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 mid-cell, 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 mid-cell,
and cells will become polarised along the long axis for large enough
reactivation length $\ell$. 


and maintenance to scale with its length. In the following section, selected, as one expects energetic costs for interface establishment important role in determining the orientation of the axis ultimately general, however, the length of the interface may also play an a single position along the interface between the PAR domains. In (interface) are enhanced at the cell poles.

The amount of cytosolic bulk proteins for each mid-cell as illustrated (bottom).

In the heuristic arguments outlined above, we tacitly considered to reattach at mid-cell. Hence, dynamics that are based on associated with the activation – aPAR-dominant state as the unpolarised state. For a typical cell size and cytosolic diffusion constants in the range of $D_{\text{cyt}} = 5–50 \mu m^2 s^{-1}$, linear stability analysis shows that second- and higher-order modes are negligible compared with the first even and odd modes, $\sigma_e$ and $\sigma_o$. In the parameter regime under consideration, 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 axis 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.

Growth rates of long versus short-axis polarisation. To put the above heuristic reasoning concerning the role of membrane-to-bulk ratio on a firm basis, we first performed a mathematical analysis in two-dimensional elliptical geometry, building on previous investigations of intracellular pattern formation.

Importantly, in the bounded geometry of a cell, broken detailed balance due to the dephosphorylation–phosphorylation cycle implies that a uniform well-mixed state can no longer be a steady state of the system. Instead, all steady states show cytosolic gradients with a density profile that is spatially non-uniform, but unpolarised. As the reactive dynamics in the PAR system is bistable, there are two such unpolarised 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 unpolarised state.

To perform a linear stability analysis with respect to this unpolarised state, we use Fourier modes specific for elliptical geometry. 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 patterns aligned along the long and short axes, respectively (Fig. 3a). If the real parts of the growth rates $\sigma$ of all modes are negative, small spatial perturbations of the unpolarised state will decay and it will remain stable. In contrast, a positive real part of any growth rate ($\sigma > 0$) indicates that the unpolarised state is unstable, and initially a pattern will emerge corresponding to the mode with the highest growth rate (Fig. 3b).

Hence, linear stability analysis can identify the parameter regime where patterns of a certain symmetry (short axis vs. long axis) form spontaneously. On very general grounds, we expect that bifurcations in mass-conserving reaction–diffusion systems are subcritical and hence these pattern attractors persist over some range outside the linear unstable parameter regime (see also details on FEM simulations in the Methods section), where patterns do not form spontaneously but can be triggered by a finite perturbation, such as the fertilisation event.

For a typical cell size and cytosolic diffusion constants in the range of $D_{\text{cyt}} = 5–50 \mu m^2 s^{-1}$, linear stability analysis shows that second- and higher-order modes are negligible compared with the first even and odd modes, $\sigma_e$ and $\sigma_o$. In the parameter regime under consideration, 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 axis 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.

Cytoplasmic reactivation length is crucial for axis selection. We computed $\delta \sigma$ as a function of $\lambda$ and $D_{\text{cyt}}$. As shown in Fig. 3c, the even mode dominates ($\delta \sigma > 0$) for large cytosolic diffusion constant and low reactivation rates (favouring long-axis polarisation), 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 the establishment of long-axis polarity. While linear stability analysis can elucidate the selection of
the polarisation axis during the onset of pattern formation, it cannot predict the final pattern as it neglects nonlinear effects in the diffusion–reaction equation. To determine the final stable polarisation axis, we performed finite-element (FEM) simulations; see also details on FEM simulations in the Methods section. These simulations show that there is a threshold value for the reactivation length $\ell^* = 11.4 \mu m$ above/below, which cells stably polarise along the long axis/short axis (Fig. 3c). We conclude that in a two-dimensional cell geometry the reactivation length $\ell$, which determines the spatial distribution of active proteins, is the decisive parameter that determines both initial axis selection and its long-term maintenance. How in full three-dimensional cell geometry this effect of the membrane-to-bulk ratio interacts with the role of the interface length will be discussed below.

**Role of phosphorylation rates.** Whether there is a spatial separation between aPAR and pPAR domains, is known to depend on the relative magnitude of the phosphorylation rates $k_{Ap}$ and $k_{pA}$. An interface between different domains exists and can be maintained only if these antagonistic phosphorylation processes are balanced. To determine the necessary conditions for this balance, we analysed the stability of the unpolarised state using linear stability analysis varying both phosphorylation rates over one order of magnitude. We fixed $D_{cyt} = 30 \mu m^2 s^{-1}$ and chose two representative reactivation rates, $\lambda = 0.3 s^{-1}$ and $\lambda = 0.05 s^{-1}$, corresponding to reactivation lengths, $\ell = 10 \mu m$ and $\ell = 24.5 \mu m$, respectively.

Our analysis in elliptical cell geometry shows that spontaneous polarisation starting from the unpolarised state arises only within a limited range of $k_{pA}/k_{Ap}$ values (cones in Fig. 4), in accordance with previous studies using a one-dimensional model. Strikingly, however, we find that the selection of the polarisation axis does not depend on the mutual antagonism, but primarily on the activation–deactivation cycle. The ratio of the phosphorylation rates mainly determines the initial preference for a polarisation axis starting from an unpolarised state (Fig. 4a, b). Specifically, we find that for $\lambda = 0.3 s^{-1}$, the first even mode grows more slowly than the first odd mode ($\delta \sigma < 0$), favouring short-axis polarisation. In contrast, for slower reactivation $\lambda = 0.05 s^{-1}$, the first even mode grows faster than the first odd mode ($\delta \sigma > 0$). These respective preferences are most pronounced for large $k_{pA}/k_{Ap}$. For the mid to low range of $k_{pA}/k_{Ap}$, one finds $\delta \sigma = 0$, i.e., linear stability analysis does not predict a clear preference for either long- or short-axis polarisation. FEM simulations (for details on the FEM simulations see the Methods section) show, however, that — irrespective of the ratio $k_{pA}/k_{Ap}$— long- and short-axis polarisation in the final steady state is obtained for $\ell = 10 \mu m$ and $\ell = 24.5 \mu m$, respectively; see Supplementary Movies M2d_1– M2d_3 and Supplementary Tables 2, 3. These simulations confirm that the reactivation length $\ell$ is the deciding factor for axis selection in elliptical geometry.

The FEM simulations further show that outside of the parameter regime of linear instability there exist stable polarised states, showing that the system is excitable, i.e., that patterns can be triggered by a large enough finite perturbation; see Supplementary Notes 1. This parameter regime is actually quite broad (see also Supplementary Fig. 1). As a generic example for an external stimulus, we have investigated how the PAR system reacts to initial concentration gradients on the membrane that were aligned along the final stable polarisation axes. We find that large enough gradients can indeed stimulate the formation of cell polarisation. It would be interesting to specify external cues more in detail experimentally and study how they affect pattern formation. In another work, we recently showed that Turing instabilities and excitability (i.e., the ability to establish a pattern by applying a larger perturbation to the stable uniform steady state) are mechanistically linked in mass-conserving systems, such as the PAR system.

Hence, even in systems where polarity is established by an external
cue, identifying a Turing instability also locates regions where external stimulation leads to stable pattern formation.

The dependence of initial growth rates on the ratio of phosphorylation rates can be attributed to the fact that, in the unpolarised (aPAR-dominant state), the cytosolic concentration of aPARs increases with the rate at which aPARs are phosphorylation rates increases with the rate at which aPARs are phosphorylated by pPARs, i.e., with a reduction in \( k_{Pa} \) and \( k_{Ap} \) (Fig. 4c, d). If a protein species is abundant in the cytosol, phosphorylated by pPARs, i.e., with a reduction in \( k_{Pa} \) and \( k_{Ap} \), the per capita rate \( k_{Ap} \) has to be increased relative to \( k_{Pa} \), the per capita rate \( k_{Ap} \) to allow for polarisation. This upward shift is easily explained, as the effective mutual phosphorylation rates are given by \( k_{Ap}[P] \) and \( k_{Pa}[A_{22}] \), respectively, where \( [A_{22}] \) is mainly limited by the availability of \( [A_{2}] \). Therefore, when the concentration of pPAR proteins \( [P] \) is increased relative to \( [A_{2}] \), 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 polarisation is possible for a broader range of \( k_{Pa}/k_{Ap} \). Increasing the concentration of the scaffold protein \( [A_{1}] \) relative to \( [A_{2}] \), which phosphorylates pPARs, decreases the lower bound of \( k_{Pa}/k_{Ap} \) that allows for polarisation. This is a consequence of the increased reservoir size of \( A_{1} \) which implies a higher rate of attachment of cytosolic \( A_{1} \) to the membrane.
Role of interface length in three-dimensional cell geometry.

With the previous analysis in two-dimensional cell geometry, we performed extensive FEM simulations, restricting ourselves to parameter regimes that we identified as most relevant from the two-dimensional geometry (see Table 4 and compare with Table 1). From a weakly perturbed unpolarised state, we observe the following time evolution (Fig. 6a, b); see the Methods section on FEM simulations for 3d system and see our Supplementary Movies 4 and 5 (M3d_1.mp4 and M3d_2.mp4). During an initial time period $T_{\text{init}}$ a protein pattern forms that is either aligned along the short or long cell axis or somewhere in between. While long-axis polarisation is stable, any other polarisation is only metastable and after some persistence time $T_{\text{pers}}$ transitions into stable long-axis polarisation during $T_{\text{trans}}$ as discussed in Supplementary Note 5 and Supplementary Figs. 4–6 there are (unphysiological) cell geometries where short-axis polarisation is stable.

We observe that, as for the two-dimensional case, initial long-axis polarisation is favoured for large cytosolic diffusion constants $D_{\text{cyt}}$ and low reactivation rates $\lambda$, while initial short-axis polarisation is favoured for the diametrically opposed case; compare Fig. 6d with Fig. 3d. This shows that the local membrane-to-bulk ratio is indeed the main factor that determines initial axis selection. Moreover, the persistence time $T_{\text{pers}}$ (Fig. 6c) and the transition time $T_{\text{trans}}$ (Fig. 6c, d) both depend strongly on $D_{\text{cyt}}$ but only weakly on $\lambda$. In the regime with a clear preference for short-axis polarisation (below the dashed line in Fig. 6d), $T_{\text{trans}}$ becomes as large as several hours; for reference see Fig. 6d with $\ell^* \approx 7\,\mu m$; for further discussion and results on time scales, see also Supplementary Note 8 and Supplementary Fig. 8.

Finally, we wanted to investigate the main factors that determine the stability of long- versus short-axis polarisation. As the essential novel feature of a three-dimensional cell geometry is the length of the interface between the PAR domains, we speculated that an additional mechanism relevant for axis polarisation is the minimisation of the interface length. To test this hypothesis, we performed FEM simulations in different prolate and oblate geometries; see Supplementary Notes 5 and Supplementary Figs. 4–6, and Supplementary Movies 6–8 (M3d_3 to M3d_5). We find that (for a given set of model parameters), the local diffusive protein fluxes from the cytosol to the membrane at the aPAR–pPAR interface are the same for short- and long-axis polarisation. Hence, the corresponding total fluxes scale with the length of the interface (see also Supplementary Note 6). This suggests that the mechanism responsible for long-axis stability is minimisation of protein fluxes. As a consequence, the transition times $T_{\text{trans}}$ from short- to long-axis polarisation should also decrease with larger cytosolic protein fluxes as the maintenance of

![Fig. 5 Relative protein numbers determine robustness of cell polarity.](image)

Linear stability analysis for a range of density ratios $\frac{[P]/[A_2]}{[A_1]/[A_2]}$: $[A_2]$ was kept constant. Each graph shows the change of the range of phosphorylation ratios $k_{P/PA}$ and relative reactivation lengths ($\ell^*$) where the balance state is linearly unstable, with $\ell^*$ given by the same colour code as in Fig. 4a; fixed parameters are $k_{P} = 0.4\,\mu m^{-1} s^{-1}$ and $D_{\text{cyt}} = 30\,\mu m^2 s^{-1}$, and further parameters not varied can be found in Table 1. FEM parameter sweeps of $k_{P}$ and $\lambda$, with fixed parameters $k_{P} = 0.4\,\mu m^{-1} s^{-1}$ and $D_{\text{cyt}} = 30\,\mu m^2 s^{-1}$, for each density set show that the steady-state polarisation axis also depends strongly on the ratio $[A_1]/[A_2]$. The steady state switches from short- to long-axis polarisation at the black line in each graph, indicating $\ell^*$.

and hence a fast local re-dimerisation of $A_2$ (which lacks an inactive phase) right after the detachment of a heterodimer $A_{12}$. This newly formed heterodimer $A_{12}$ is then competent to phosphorylate pPARs. Thus it is plausible that even for low $k_{P/PA}$, one can achieve a balance of mutual antagonism, extending the lower bound of the polarisation regime. Second, changing the ratio $\frac{[A_1]/[A_2]}{[A_1]/[A_2]}$ 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 $[A_2]$ (Fig. 5). Again, this can be understood as a reservoir effect: globally abundant $A_2$ promotes immediate re-dimerisation of $A_2$ with any available $A_1$. Axis selection is then affected by the polar recycling of $A_3$.

Taken together, both of these findings emphasise the importance of the activation–deactivation cycle. A cell polarises more robustly when amounts of scaffold proteins are higher. However, at the same time, the cytosolic reactivation length has to increase significantly in order to also robustly maintain long-axis polarisation.

Fig. 5 Relative protein numbers determine robustness of cell polarity.

### Note

4) One would at first sight expect the same conclusions to hold. However, there is a fundamental difference between a three- and a two-dimensional cell geometry. While for an ellipse the interface is always point-like, for a prolate spheroid the interface is longer for short-axis polarisation than for long-axis polarisation; in our case, we have 135 $\mu m$ and 94 $\mu m$, respectively (Fig. 1d). This inherent difference between a two- and a three-dimensional cell geometry could significantly affect the protein dynamics on the membrane and in the cytosol. In the absence of an interface, the only geometric effect is the membrane-to-bulk ratio. Therefore, as in the two-dimensional case, we expect this ratio to be the main factor that determines the initial formation of the protein domains and the interface between them. However, as soon as an interface has formed, its length is likely to affect the stability of the polarisation axis. The maintenance of the interface between protein domains is presumably energetically costly (protein fluxes sustaining antagonistic reactions, reactivation and rebinding have to be maintained). Therefore, since the interface is longer for short-axis than for long-axis polarisation, it is possible that even an initially favoured alignment of polarisation with the short axis can become unstable.

To assess the protein dynamics of the system in full cell geometry, we performed extensive FEM simulations, restricting ourselves to parameter regimes that we identified as most relevant from the two-dimensional geometry (see Table 4 and compare with Table 1). Starting from a weakly perturbed unpolarised state, we observe the following time evolution (Fig. 6a, b); see the Methods section on FEM simulations for 3d system and see our Supplementary Movies 4 and 5 (M3d_1.mp4 and M3d_2.mp4). During an initial time period $T_{\text{init}}$, a protein pattern forms that is either aligned along the short or long cell axis or somewhere in between. While long-axis polarisation is stable, any other polarisation is only metastable and after some persistence time $T_{\text{pers}}$, transitions into stable long-axis polarisation during $T_{\text{trans}}$ as discussed in Supplementary Note 5 and Supplementary Figs. 4–6 there are (unphysiological) cell geometries where short-axis polarisation is stable.

We observe that, as for the two-dimensional case, initial long-axis polarisation is favoured for large cytosolic diffusion constants $D_{\text{cyt}}$ and low reactivation rates $\lambda$, while initial short-axis polarisation is favoured for the diametrically opposed case; compare Fig. 6d with Fig. 3d. This shows that the local membrane-to-bulk ratio is indeed the main factor that determines initial axis selection. Moreover, the persistence time $T_{\text{pers}}$ (Fig. 6c) and the transition time $T_{\text{trans}}$ (Fig. 6c, d) both depend strongly on $D_{\text{cyt}}$ but only weakly on $\lambda$. In the regime with a clear preference for short-axis polarisation (below the dashed line in Fig. 6d), $T_{\text{trans}}$ becomes as large as several hours; for reference see Fig. 6d with $\ell^* \approx 7\,\mu m$; for further discussion and results on time scales, see also Supplementary Note 8 and Supplementary Fig. 8.

Finally, we wanted to investigate the main factors that determine the stability of long- versus short-axis polarisation. As the essential novel feature of a three-dimensional cell geometry is the length of the interface between the PAR domains, we speculated that an additional mechanism relevant for axis polarisation is the minimisation of the interface length. To test this hypothesis, we performed FEM simulations in different prolate and oblate geometries; see Supplementary Notes 5 and Supplementary Figs. 4–6, and Supplementary Movies 6–8 (M3d_3 to M3d_5). We find that (for a given set of model parameters), the local diffusive protein fluxes from the cytosol to the membrane at the aPAR–pPAR interface are the same for short- and long-axis polarisation. Hence, the corresponding total fluxes scale with the length of the interface (see also Supplementary Note 6). This suggests that the mechanism responsible for long-axis stability is minimisation of protein fluxes. As a consequence, the transition times $T_{\text{trans}}$ from short- to long-axis polarisation should also decrease with larger cytosolic protein fluxes as the maintenance of
Fig. 6 Cell polarisation in three dimensions. a Image series from FEM (Comsol) simulation for $D_{\text{cyt}} = 6 \mu m^2 s^{-1}$, and reactivation rate $\lambda = 0.21 s^{-1}$. The series illustrates the different times which are further analysed: the time from the initial aPAR-dominated unpolarised state to the initial short-axis polarisation, $T_{\text{initial}}$; the time duration of persistent short-axis polarisation, $T_{\text{pers}}$, and the time the pattern takes to turn from short- to long-axis polarisation, $T_{\text{trans}}$. b The angle $\Theta$ of the concentration maximum of membrane-bound $A_1$ is plotted against simulation time for different $D_{\text{cyt}}$, as indicated in the graph. c $T_{\text{initial}}$, $T_{\text{pers}}$, $T_{\text{trans}}$, plotted as a function of $D_{\text{cyt}}$ for $\lambda = 0.09 s^{-1}$ and $\lambda = 0.3 s^{-1}$. d The magnitude of the transition time from short- to long-axis polarisation, $T_{\text{trans}}$, in $D_{\text{cyt}}$-$\lambda$ parameter space; a cell was considered to be polarised along the short axis if $90° - 10° \leq \Theta \leq 90° + 10°$. The monochrome cyan-coloured region above the grey line corresponds to a parameter region where there is no short-axis polarisation, but the polarisation axis is aligned along the diagonal or long axis from the beginning. The dashed lines demarcate parameter regimes where the initial polarisation is aligned perfectly with the short axis ($\Theta = 90°$) or with the long axis, as indicated in the graph.

Discussion

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

Polarisation in C. elegans is controlled by several mechanisms and their interplay: an initial polarisation cue of the centrosome, contraction of the actomyosin network and the PAR reaction–diffusion system which leads to polarisation in a self-organised manner, but also interacts with the centrosome as well as with the actomyosin network. Recent research has further revealed some redundant pathways for the reaction–diffusion system depending on other proteins, such as CHIN-1, LGL-1 and Cdc-4211,17,40,41. In view of this complexity, it is constructive to disentangle all individual building blocks, as well as kinetic, and investigate each separately in order to properly identify the underlying mechanisms which (i) leads to polarisation and (ii) aligns it with the long axis. With our work, we could now shed light on polarisation and its alignment by the PAR reaction–diffusion system in 2d and in 3d. We expect the insights gained to be essential elements for a future three-dimensional model which combines the reaction–diffusion system with mechanical effects to quantitatively understand pattern formation in the C. elegans embryo.

Previous experiments supported by mathematical models in simplified cell geometry have indicated that balance between mutual phosphorylation of aPAR and pPAR proteins is a key mechanism responsible for cell polarisation.8,15,16,42 Our theoretical results in realistic cell geometry support this finding. In addition, we have shown that 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-4224). 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 processes for axis selection are cytosolic, specifically the cytosolic diffusion and an inactive (phosphorylated) phase of PAR-3 and PAR-2 after detachment from the membrane. The reactivation time $(\lambda^{-1})$ implies a cytosolic reactivation length $\ell = \sqrt{D_{\text{cyt}}/\lambda}$, which defines a cytosolic zone of inactive proteins close to the membrane. Proteins with a short 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 the PAR-6 PKC-3 complex, are available for rebinding immediately after detachment from the membrane and are thus strongly caged to the cell poles.

Our theoretical analysis in a two-dimensional elliptical geometry shows that only for a sufficiently large cytosolic reactivation length $\ell$ does the long axis become the preferred polarisation axis, at onset as well as for the steady state. For the onset of polarisation, starting from a spatially homogeneous protein distribution, this result is fully transferable to a three-dimensional prolate spheroid. However, in such a realistic cell geometry, the length of the aPAR–pPAR interface also becomes important for the stability of the polarisation axis. Our simulation results suggest an (approximate) extremal principle: The dynamics tries to
minimise the interface length such that for physiologically relevant geometries the long axis is always stable. Initial metastable short-axis polarisation is observed if the reactivation length $\ell$ is small (fast reactivation), such that proteins exhibit caging at the polar zones. In that regime, the transition times from short-axis to long-axis polarisation can be of the order of several hours. In contrast, if $\ell/L \geq 0.3$, this time can be as short as 10 min. This implies that without guiding cues the reaction–diffusion system requires a sufficiently slow phosphorylation–dephosphorylation cycle and a sufficiently large diffusion constant for fast and robust long-axis polarisation.

Furthermore, how slow reactivation and how fast cytosolic diffusion need to be in order to efficiently and robustly establish and maintain long-axis polarisation, depends on the ratio of PAR-3 proteins to the PAR-6 PKC-3 complex: a larger cytosolic pool of PAR-3 attenuates the effect of selecting the interface at midplane and at the same time strengthens the tendency of PKC-3 to put the interface at the poles. Hence, we predict that increasing the number of PAR-3 should destabilise long-axis polarisation in favour of short-axis polarisation.

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. We expect the following findings to be generic for mass-conserved intracellular protein systems: local membrane-to-bulk ratio and the length of interfaces between different protein domains act as geometric cues for protein pattern formation, and an activation–deactivation as well as 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.

**Methods**

**Model.** First we introduce and discuss the mathematical formulation and analysis of the reaction–diffusion model for PAR protein dynamics. To account for a realistic cell geometry we use, similar as in previous studies of the Min system\(^24\), a two-dimensional elliptical geometry where the boundary of the ellipse ($\partial \Omega$) represents the membrane and the interior ($\Omega$) represents the cytosol. Attachment–detachment processes are encoded by nonlinear reactive boundary conditions as introduced in ref.\(^{24}\). Protein interactions are assumed to be bimolecular from which follow mass-action law kinetics. In the following section, a species identifies a mass-conserved protein type, whereas a component indicates the subgroup of proteins in a specific state, such as e.g., ‘phosphorylated’ (‘inactive’) or ‘membrane bound’.

**Cytosolic dynamics.** Proteins in the cytosol are all assumed to diffuse with the same diffusion constant, $D_{\text{cyt}} = 30 \mu m^2 s^{-1}$ (see also Table 1). In addition, we consider dephosphorylation (reactivation) of phosphorylated proteins with an activation (dephosphorylation) rate $\lambda = 0.05 / s$ (see also Table 1). The cytosolic concentration of each protein type $X$ is denoted by $c_X$ in its active form and by $c_{\overline{X}}$ in its inactive form (if applicable). The dynamics of the bulk components are thus given by the following set of reaction–diffusion equations:

$$\partial_t c_{\overline{X}} = D_{\text{cyt}} \nabla^2 c_{\overline{X}} + \lambda c_X,$$  

$$\partial_t c_X = D_{\text{cyt}} \nabla^2 c_X - \lambda c_X,$$  

$$\partial_t c_{\overline{A}} = D_{\text{mem}} \nabla_{\parallel}^2 c_{\overline{A}},$$  

$$\partial_t c_A = D_{\text{mem}} \nabla_{\parallel}^2 c_A + \lambda c_{\overline{A}},$$  

$$\partial_t m_X = D_{\text{mem}} \nabla_{\parallel}^2 m_X + k^{\text{off}}_{A} c_X - k^{\text{on}}_{A} c_{\overline{A}} m_A - k_{Ap} m_P m_X - k_{\text{off}} m_A c_{\overline{A}} c_X,$$  

$$\partial_t m_P = D_{\text{mem}} \nabla_{\parallel}^2 m_P + k^{\text{off}}_{P} m_A - k^{\text{on}}_{P} c_{\overline{X}} m_P ,$$  

$$\partial_t m_{\overline{A}} = D_{\text{mem}} \nabla_{\parallel}^2 m_{\overline{A}} - k^{\text{off}}_{A} c_X + k^{\text{on}}_{A} c_{\overline{A}} m_A - k_{Ap} m_P m_{\overline{A}},$$  

$$\partial_t m_{\overline{P}} = D_{\text{mem}} \nabla_{\parallel}^2 m_{\overline{P}} + k^{\text{off}}_{P} m_A - k^{\text{on}}_{P} c_{\overline{X}} m_P,$$

where $\nabla^2$ is the Laplacian in the two-dimensional bulk.

**Membrane dynamics.** On the membrane, all species are assumed to diffuse with the respective diffusion constant, $D_{\text{mem}} = 0.28 \mu m^2 s^{-1}$ and $D_{\text{mem}} = 0.15 \mu m^2 s^{-1}$ for $\alpha$PARs and $\delta$PARs (see also Table 1). With $m_P$, we denote the membrane-bound concentration of protein $X$. Then, the bimolecular reactions discussed above (see Fig. 1) translate into the following set of reaction–diffusion equations:

$$\partial_t m_{\overline{A}} = D_{\text{mem}} \nabla \nabla_{\parallel}^2 m_{\overline{A}} + k^{\text{off}}_{A} c_X - k^{\text{on}}_{A} c_{\overline{A}} m_A - k_{Ap} m_P m_{\overline{A}} - k_{\text{off}} m_{\overline{A}} c_{\overline{A}} c_X,$$  

$$\partial_t m_{\overline{P}} = D_{\text{mem}} \nabla \nabla_{\parallel}^2 m_{\overline{P}} + k^{\text{off}}_{P} m_A - k^{\text{on}}_{P} c_{\overline{X}} m_{\overline{P}},$$  

where $\nabla^2$ is the Laplacian operator on the boundary $\partial \Omega$, i.e., on the membrane.

**Reactive boundary conditions.** The membrane dynamics and cytosolic dynamics are coupled through reactive boundary conditions. These describe the balance between diffusive fluxes ($D_{\text{mem}} \nabla_{\parallel} c_X$ acting on cytosolic concentration) and attachment and detachment processes between the membrane and cytosol:

$$D_{\text{mem}} \nabla_{\parallel} c_X = k^{\text{off}}_{A} (m_A + m_{\overline{A}}) c_X - k^{\text{on}}_{A} c_{\overline{A}} m_A,$$  

$$D_{\text{mem}} \nabla_{\parallel} c_A = \left( k_{Ap} m_P + k^{\text{on}}_{P} c_{\overline{X}} \right) m_A - k_d c_A m_A,$$  

$$D_{\text{mem}} \nabla_{\parallel} c_P = k^{\text{off}}_{P} m_A - k^{\text{on}}_{P} c_{\overline{X}} m_P,$$  

$$D_{\text{mem}} \nabla_{\parallel} m_X = k_{Ap} m_P m_A + m_A,$$  

$$D_{\text{mem}} \nabla_{\parallel} m_P = k_{Ap} m_P m_A,$$

where $\nabla_{\parallel}$ is the Nabla operator perpendicular to the boundary, such that $D_{\text{mem}} \nabla_{\parallel} c_X$ is the flux operator between the cytosol and membrane.

**Mass conservation.** On the time scale of establishment and maintenance of polarisation in C. elegans, PAR protein production and degradation are negligible. Hence, the total number $N_X$ of each protein species $X \in \{A, \overline{A}, P\}$ is conserved. It can be obtained by integrating the average densities over the whole space or by integrating the space-dependent cytoplasmic concentrations and membrane concentrations over $\Omega$ and $\partial \Omega$, respectively:

$$N_A = \int_{\Omega} \rho_A = \int_{\partial \Omega} c_A + \int_{\Omega} (m_A + m_{\overline{A}}),$$  

$$N_{\overline{A}} = \int_{\partial \Omega} \rho_{\overline{A}} = \int_{\Omega} c_{\overline{A}} + \int_{\Omega} m_{\overline{A}},$$  

$$N_P = \int_{\partial \Omega} \rho_P = \int_{\Omega} (c_P + c_{\overline{X}}) + \int_{\Omega} m_P,$$

where $\rho_A$ and $\rho_{\overline{A}}$ denote integrals over the interface and the boundary of the ellipsoid, respectively.

**Linear stability analysis.** In the following section, we outline the main steps required to perform a linear stability analysis (LSA) in elliptical geometry, by emphasising the major differences relative to the well-known stability analysis in planar system geometries with no bulk-boundary coupling (see e.g., a didactic derivation of linear stability analysis written by Cross and Greenside\(^{23}\)). A detailed derivation of LSA in elliptical geometry can be found in the Supplementary Information of Halatek et al.\(^{37}\).

**Reaction–diffusion equations in elliptical geometry.** A LSA yields the initial dynamics of a system perturbed from any of its steady states. In the context of pattern formation in reaction–diffusion systems, this is typically a uniform steady state. The eigenfunctions of the linearised system (around the steady state) serve as an orthogonal basis, in which any perturbation can be expressed. Planar systems, these are simply Fourier modes, e.g., $- \cos(qx) \cdot \cos(qy)$, with spatial variable $x$ and wave-number $q$, where $q$ is chosen such that boundary conditions are satisfied. The LSA then yields the temporal eigenvalues $\sigma_q$ (growth rates) for each wavenumber that express exponential growth or decay, and possible oscillation (if the imaginary part $\Im(\sigma_q) \neq 0$) of the respective eigenfunction $\exp(\sigma_q t) \cos(qx)$. Hence, the main objective is (i) to derive the eigenfunctions for the linearised system in the corresponding geometry, and (ii) to calculate the associated growth rates (real parts $\Re(\sigma_q)$), where positive growth rates signify formation of patterns with wavelength $\approx 1/q$.

For reaction–diffusion systems with bulk-boundary coupling in elliptical geometry, there are three major complications with this approach.

Due to bulk–boundary coupling, we are faced with two separate sets of reaction–diffusion equations. One set is defined in the bulk and accounts for the dynamics in the cytosol. Here, reactions are assumed to be linear (first-order kinetics) and typically account for nucleotide exchange or (de-)phosphorylation, Eqs. (3)–(7). The second set is defined on the boundary and accounts for the dynamics on the membrane (or cell cortex), including diffusion and reaction, Eqs. (8)–(10).
The first complication arises as follows: given orthogonal elliptical coordinates
\[ x = d \cos \mu \cos \nu, \quad y = d \sin \mu \sin \nu, \]
with \( \nu \) \( \mu \) varying, \( \delta \delta \) \( 2 \nu \), and elliptical eccentricity
\[ d = \sqrt{a^2 - b^2} \]
with (long half-axis \( a \) and short half-axis \( b \)), the diffusion operator in the bulk \( D_{\text{cyt}} \) reads:
\[ \frac{1}{d} \left[ \sinh^2 \mu + \sin^2 \nu \right] \frac{\partial^2}{\partial \mu^2} + \frac{1}{d} \left[ \sinh^2 \mu + \sin^2 \nu \right] \frac{\partial^2}{\partial \nu^2} \phi. \] (21)

On the boundary the diffusion operator \( D_{\text{mem}} \) acts along constant \( \mu = \mu_0 = \arctan(b/a) \) and reads:
\[ D_{\text{mem}} \left( \cos \nu \sin \nu \frac{\partial}{\partial \nu} + \sin \nu \frac{\partial}{\partial \nu} \phi + \frac{1}{d} \left[ \sinh^2 \mu + \sin^2 \nu \right] \frac{\partial^2}{\partial \nu^2} \phi \right). \] (22)

Due to these different diffusion operators, the sets of reaction–diffusion equations in the bulk and on the boundary do not share the same set of canonical eigenfunctions (i.e., eigenfunction obtained from separation of variables). To overcome this problem the diffusion on the membrane can be more conveniently expressed in archlength parametrisation \( s(\nu) \):
\[ s(\nu) = \frac{1}{d} \left[ \sinh^2 \mu + \sin^2 \nu \right] \frac{\partial \nu}{\partial \nu} \phi. \] (23)

Then, the diffusion operator \( D_{\text{mem}} \) simplifies to \( D_{\text{mem}} \phi \), and the eigenfunctions are obtained as
\[ \Psi_{\epsilon \mu} \left( \mu_0, s(\nu) \right) = \cos \left( \frac{2\pi}{\lambda} s(\nu) \right), \] (24)
\[ \Psi_{\epsilon \mu} \left( \mu_0, s(\nu) \right) = \sin \left( \frac{2\pi}{\lambda} s(\nu) \right). \] (25)

with the circumference of the ellipse \( S = 2\pi \). The goal is then to express these functions in terms of the orthogonal eigenfunctions of the bulk problem—the Mathieu functions, here denoted by \( \Psi(\nu) \) and \( R(\mu) \)—which are obtained as solutions of the Mathieu equations:
\[ 0 = \frac{d^2}{d\nu^2} \Psi(\nu) + \left( a - 2q \cdot \cos(2\nu) \right) \Psi(\nu), \] (26)
\[ 0 = \frac{d^2}{d\mu^2} R(\mu) + \left( -a \cos (2\mu) \right) \frac{d^2}{d\mu^2} R(\mu). \] (27)

Here \( a \) is a constant of separation, and
\[ q = \frac{1}{2} \left( a + \lambda \right)^2 4D_{\text{cyt}}. \] (28)

denotes a dimensionless parameter (not to be confused with a wavenumber!). For small \( q \), analytical approximations of the Mathieu functions can be obtained\(^4\) and matched with the eigenfunctions \( \Psi_{\epsilon \mu} \) and \( \Psi_{\epsilon \mu} \) at the boundary \( \mu = \mu_0 \).

The second complication is a consequence of the coupling between bulk and boundary processes through the reactive boundary condition, see e.g., the model equations Eqs. (11)–(15). This coupling introduces an explicit dependence of the linearised system on the (derivative of the) radial eigenfunctions \( R(\mu) \) (see ref. \(^2\)), which, in turn, depends on the temporal eigenvalues \( \sigma \) in a non-algebraic fashion. Usually, the final step in any LA is the solution of a characteristic equation \( \sigma = f(\sigma) \), which is typically polynomial in \( \sigma \). Due to the bulk-boundary coupling, this is no longer the case (irrespective of the geometry, see e.g., ref. \(^2\)); the characteristic equation is transcendental and can only be solved numerically for each parameter combination\(^3\). Therefore, it is not possible to derive a general stability criterion analogous to that known for planar systems without bulk-boundary coupling.\(^3\) We further note that the boundary condition introduces a coupling between the angular eigenfunctions \( \Psi(\nu) \), which, however, is small and can be neglected\(^3\).

The final complication arises as consequence of the cytosolic reaction cycle. This cycle generically precludes the existence of a uniform steady state (including states uniform along the boundary). The origin of this symmetry adaption process has been discussed in ref. \(^3\). Following ref. \(^3\), we approximate the near-uniform steady state with the eigenfunction that is constant along the boundary, i.e., \( \Psi_{\epsilon \mu} \left( \mu_0, s(\nu) \right) \). In this case, nonlinearities (which are restricted to the boundary) do not induce mode coupling, which would otherwise complicate the LSA.

Finite-element simulations (FEM). Linear stability analysis can only predict the onset of pattern formation. In order to understand the full nonlinear protein dynamics and to determine the steady states corresponding to given parameter sets, we further performed finite-element (FEM) simulations on a triangular mesh using Comsol Multiphysics 5.1–5.4 (upgrading versions).

Setup for FEM simulations. As time-dependent solver in Comsol Multiphysics, we chose PARDISO with a multithreaded nested iteration. The time stepping was performed with a relative tolerance of \( 10^{-6} \) between time steps and solved with a multistep method (BDF). In all simulations, we used triangular meshing (setting ‘Solver’ with additional refinement at the boundary, i.e., along the membrane. As for the linear stability analysis, if not specified otherwise, the parameters for the FEM simulations can be found in Table 1. For the standard parameter sets given in Table 1, we ran the simulation up to \( 5 \times 10^4 s \). Since the system reached the steady state for most parameter sets at the latest after \( 5 \times 10^3 s \), we limited simulation times for large parameter sweeps at \( 10^4 s \).
FEM simulations for 3d system. In 3d FEM, simulations for all sweeps were initiated with an initial PAR-dominant concentration on the membrane and 1% random perturbation thereof. All parameters are shown in Table 4. For the sweep of λ versus Dmem resulting in the data discussed in the main text and Fig. 6, the parameter range was set to Dmem = 2–32 μm² s⁻¹ in steps of 2 μm² s⁻¹, and reactivation rate λ = 0.03–0.3 s⁻¹ in steps of 0.03 s⁻¹. The full region of the formation of any pattern can be found by using the feature that the absolute value of membrane gradients is zero for a homogeneous distribution on the membrane and a positive number for inhomogeneous (patterned) protein distributions on the membrane. To distinguish between long and short-axis patterns the FEM simulations were analysed by investigating (i) the angle of the concentration maxima on the membrane and in ellipsoidal coordinates (which is 90° for perfect short-axis polarization and 0/180° for perfect long-axis polarization) and additionally (ii) the distance between the concentration maxima on the membrane. To numerically investigate the onset of long-axis polarization, which is very sensitive to λ, a finer sweep was additionally performed with Dmem = 2–32 μm² s⁻¹ in steps of 2 μm² s⁻¹, and reactivation rate λ = 0.015–0.01 s⁻¹ in steps of 0.005 s⁻¹. To find the boundary for a polarity onset with long-axis alignment, we filtered for a short axis and a diagonal onset.

Table 4 Parameters for three-dimensional FEM simulations.

| Parameters | α[μm] | b[μm] | Dmem[μm² s⁻¹] | k PAR[μm⁻² s⁻¹] | k PAB[μm⁻¹ s⁻¹] | k PAR[μm² s⁻¹] | k PAB[μm² s⁻¹] | k PAR[μm² s⁻¹] |
|------------|-------|-------|---------------|-----------------|----------------|----------------|----------------|----------------|
| 27         | 15    | 30    | 0.034         | 0.28            | 0.15           | 0.3            | 8.0            | 2.5            |
| For the sweeps shown in Fig. 6, all parameters but λ and Dmem were chosen as shown in this Table. λ was varied between 3 × 10⁻² s⁻¹ and 0.3 s⁻¹ with a uniform spacing of 3 × 10⁻² s⁻¹. Dmem was varied from 2 μm² s⁻¹ to 32 μm² s⁻¹ with a uniform spacing of 2 μm² s⁻¹.

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Competing interests
The authors declare no competing interests.

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