A cell-size threshold limits cell polarity and asymmetric division potential

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Reaction–diffusion networks underlie pattern formation in a range of biological contexts, from morphogenesis of organisms to the polarization of individual cells. One requirement for such molecular networks is that output patterns be scaled to system size. At the same time, kinetic properties of constituent molecules constrain the ability of networks to adapt to size changes. Here, we explore these constraints and the consequences thereof within the conserved PAR cell polarity network. Using the stem-cell-like germ lineage of the Caenorhabditis elegans embryo as a model, we find that the behaviour of PAR proteins fails to scale with cell size. Theoretical analysis demonstrates that this lack of scaling results in a size threshold below which polarity is destabilized, yielding an unpolarized system. In empirically constrained models, this threshold occurs near the size at which germ lineage cells normally switch between asymmetric and symmetric modes of division. Consistent with cell size limiting polarity and division asymmetry, genetic or physical reduction in germ lineage cell size is sufficient to trigger loss of polarity in normally polarizing cells at predicted size thresholds. Physical limits of polarity networks may be one mechanism by which cells read out geometrical features to inform cell fate decisions.

Specification of the germline in Caenorhabditis elegans begins with polarization of the zygote, P0, which initiates the first of a series of four consecutive asymmetric divisions. At each division, beginning with P0 and continuing through its germline (P lineage) descendants P1, P2 and P3, germline determinants must be sequestered within the single P lineage daughter cell (Fig. 3a). Because there is no cell growth between divisions and each cell division is unequal in both size and fate, each P lineage daughter is less than half the size of its parent. The final division of the P lineage, that of P4, is symmetric, giving rise to the two germine founder cells Z2/Z3 (refs. 1,2). How this switch between asymmetric and symmetric modes of division is regulated remains poorly understood.

Polarization of P0 depends on the PAR (partitioning-defective) proteins, which make up a self-organizing network that regulates cell polarity across metazoans3–5. Polarization is initiated by a temporal programme of PAR network activation coupled to deployment of two semiredundant cues, resulting in the formation of two opposing PAR domains that define a single polarity axis6–9. One domain is enriched in anterior or aPAR proteins (PAR-3, PAR-6, PKC-3 and CDC-42) and defines what will become the somatic daughter, while the other, enriched in posterior or pPAR proteins (LGL-1, PAR-2, PAR-1 and the CDC-42 GTPase-activator protein CHIN-1), defines what will become the P lineage daughter, which retains germ-line fate10–13. Each set of PAR proteins excludes the other from its respective domain through a set of mutually antagonistic feedback reactions. Due to diffusion of PAR proteins at the membrane, the interface between domains is characterized by opposing gradients. Such behaviour is consistent with predictions from theoretical reaction–diffusion models based on experimental measurements14–16.

Theoretical models for cell polarity typically combine local activation or recruitment of factors at a polarity site in the cell with suppression of these factors elsewhere to ensure a single axis of polarity. Prototypical examples of such networks are so-called activator–inhibitor systems, in which a slowly diffusing ‘activator’ promotes its own production within a local peak while at the same time producing a fast moving ‘inhibitor’, which suppresses formation of additional peaks elsewhere in the system17–21. Several reaction–diffusion models have been proposed to underlie cell polarity in different contexts, including local excitation–global inhibition, wave-pinning and substrate depletion models22–24. Regardless of detailed mechanism, these models exhibit characteristic length scales that emerge from the kinetic parameters of their constituent molecules, which define characteristics such as the size, extent or spacing of morphological features. For polarizing systems, these length scales must be tuned to the size of the cell to ensure the formation of a single, delimited peak that marks the polarity axis.

Here we explore the link between the size of a cell and its ability to polarize, demonstrating that a general lack of scaling of the kinetic behaviours of polarity components results in a cell-size-dependent polarity switch, which we propose limits asymmetric division potential in the C. elegans P lineage.

System-size-independent boundary gradients

To explore how cell polarity networks respond to changes in cell size, we focused on several prototypical reaction–diffusion models. These included Turing-like systems as put forth by Goryachev and Pohkilko (GOR)20 and Otsuji et al. (OT)24, wave pinning (WP)22 and a two-component reciprocal feedback model inspired by the PAR polarity network (PAR)21,25. To simplify analysis for the PAR network, we assumed symmetric rates and dosages. These systems rely on mass conservation and limiting pools of components, interconversion between active membrane-associated and inactive cytoplasmic states, and autocatalytic feedback loops, but differ in the precise form of feedback between species. For example, while GOR and WP

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Fig. 1 | The boundary interface in cell polarity models is defined by diffusive behaviour, not cell size. a, Reaction scheme for polarity models (GOR, WP) based on a single species that interconverts between active (A*) and inactive states (A). Polarity relies on positive feedback in which A* locally recruits and activates A from a rapidly diffusing cytoplasmic pool. b, Reaction scheme for a two-component polarity model based on two mutually antagonistic species that interconvert between active, membrane-bound (A*/P*) and rapidly diffusing inactive cytoplasmic states (A/P). c–f, Sample steady-state distributions reached in various polarity models for varying D = 0.025, 0.1, 0.2 μm² s⁻¹. Shaded triangles illustrate λ for each model in the slowest-diffusion case. g, Linear dependence of λ on √D. h, Linear dependence of λ on 1/√α, where α is a scaling factor applied to all reaction rates in the system. i, When system size L is reduced, λ occupies an increasing fraction of the system (λ/L), highlighting the general lack of scaling in these models.

Experimental analysis of the PAR system in C. elegans[1]. When scaling all reaction rates by a common scaling factor α, λ varied linearly with √α⁻¹ (Fig. 1h), while varying individual reaction parameters yielded more complicated relationships due to changes in gradient shape (Supplementary Fig. 1).

In contrast to this dependence on reaction and diffusion rates, λ failed to scale with system size. Consequently, as system size changed, the resulting distribution pattern of polarity components across the cell did not scale with cell size, with λ occupying an increasing fraction of the cell as the cell became smaller (Fig. 1i).

A cell-size threshold for cell polarity
Due to lack of scaling, if the system becomes small enough, the dissipative effects of diffusion will dominate, the distributions of polarity components will become uniform, and a stable polarized state will no longer be possible. To identify a minimal system size in each
Thus, consideration of the interplay between the effects of membrane diffusion of polarity components and system size suggests a simple mechanism by which cell size can induce size-dependent switching between a state that can maintain polarity and one that cannot, thereby limiting a cell’s capacity for asymmetric division at a defined size threshold (Fig. 2f).

A lack of PAR gradient scaling in vivo

We next determined whether this behaviour could explain the division pattern in the P lineage. As in P0, asymmetric division of the remaining asymmetrically dividing P lineage cells (P1, P2 and P3) is associated with PAR protein asymmetry (Fig. 3a). We confirmed that pPAR protein PAR-2 was localized to a single domain that defined what would become the germinal daughter in the subsequent division16, and this polarized distribution was sensitive to inhibition of the anterior kinase PKC-3 (refs. 17,18) (Supplementary Fig. 2 and Supplementary Video 2). Thus, P lineage cells up to and including P3 exhibit PAR protein-dependent polarity that follows the general paradigm defined for P0.

We next examined how the behaviour of the PAR network changed with system size. Despite polarity being qualitatively similar in different P lineage cells, the shape of PAR-2 concentration profiles across the cell varied (Fig. 3b,c). In the larger P0 and P1 cells, anterior and posterior domains exhibited extended plateaus of low and high PAR-2 concentration at the anterior and posterior, respectively, separated by a clearly defined interface region. In the smaller P2 cell, plateaus were less clear and more of the cell was occupied by the interface. Finally, in the smallest polarized cell of the P lineage, P3, the interface occupied nearly the entire cell, with only a very small plateau visible. Thus, as cells become smaller, the PAR boundary interface separating anterior and posterior domains takes up an increasing fraction of the cell, consistent with the behaviour of theoretical models and a general lack of scaling.

We next sought to directly manipulate cell size in vivo by altering embryo size19. Mutation of C27D9.1 or its depletion by RNA interference (RNAi), hereafter C27D9.1, increases embryo size, while RNAi targeting ima-3 reduces size; together these yield an approximate twofold range of cell sizes with circumferences spanning approximately 80–170 μm (wild type is approximately 140 μm).

To quantify the width of the boundary interface, hereafter ‘interface width’, as a function of cell size, we measured the distribution of PAR-2 and PAR-6 along the membrane in wild-type, C27D9.1 and ima-3 embryos (Fig. 3d–g; see Methods and Supplementary Fig. 3). Plotting embryo size versus interface width, we observed a modest correlation between interface width and embryo size for PAR-2, and no effect of cell size on interface width for PAR-6 over the size range examined (Fig. 3e,g). These data suggest that the PAR-2 concentration profile may sharpen somewhat in smaller cells; however, the interface width was not maintained at a fixed proportion to cell size. Consequently, for both PAR-2 and PAR-6, the interface occupied an ever larger fraction of cells as they became smaller, consistent with the lack of scaling of the PAR-2 interface observed in P lineage cells (Fig. 3b,c).

Previous work reported that the interface width of the PAR boundary is directly related to the diffusion and lifetime of PAR proteins on the membrane19. We therefore explicitly measured whether these kinetic behaviours of PAR proteins scaled with cell size, including both $D$ and off rate (dissociation rate of particles from the membrane) $k_{\text{off}}$.

To measure diffusion of PAR-2 and PAR-6, we used single-particle tracking to extract cumulative step size distributions, which matched well under all conditions, including C27D9.1 P1 cells (Fig. 4a,b). We further estimated diffusion coefficients as a function of cell circumference based on fits of mean squared displacement for each cell examined. Again, this analysis failed to yield a significant trend for either protein (Fig. 4c,d).

Fig. 2 | Membrane diffusion imposes a minimum cell-size threshold for stable polarization. a–d, Polarity across parameter space defined by $L$ and the pool (OT/GOR/WP) or ratio of pools (PAR) of available species. All exhibit a region of parameter space (grey) that permits maintenance of polarity, which is bounded by a CPSS (dashed lines). The insets show schematic representations of the steady state (polarized or unpolarized). For the PAR system, whether A or P is the dominant membrane species in the unpolarized state is colour coded. e, CPSS varies linearly with $\sqrt{D}$ for all models. f, Conceptual model for a cell-size-induced polarity switch in a stem-cell-like lineage. A stem cell polarizes and divides asymmetrically to generate another stem cell and a differentiating cell. Absent cell growth, the stem cell becomes smaller at each division. If cell size limits polarization, at some point the stem cell will fail to polarize, leading to symmetric division.

model, we explored the parameter space defined by cell size and the pool(s) of available components. Through numerical solution of the underlying equations beginning with a polarized state, we found that a cell-size threshold existed in all cases, below which the systems were unable to sustain polarity (Fig. 2a–d and Supplementary Video 1). We termed this the critical polarizable system size (CPSS). CPSS was directly proportional to the square root of diffusion of active species on the membrane (Fig. 2e). The precise relationship between CPSS and diffusion differs somewhat between models, and becomes more complex for systems with multiple membrane-bound species with differing diffusivities such as the PAR model. In the PAR model, reducing the diffusion of a single membrane species modestly reduced CPSS even if diffusion of the other was held constant, but CPSS did not scale with the slower species, meaning that the kinetic behaviour of both species must be linked to cell size to achieve scaling of CPSS (Supplementary Fig. 1).
Fig. 3 | PAR boundary gradients fail to scale with cell size. a, Schematic of PAR protein localization in P lineage cells P0, P1, P2 and P3 (pPAR, cyan; aPAR, red). In each of these cells, PAR proteins set up a cytoplasmic MEX gradient (green) that drives asymmetric segregation of germline fate determinants (orange) into a single P lineage daughter cell. The final P lineage cell, P4, divides symmetrically to yield the germline stem cells Z2/Z3. See Supplementary Video 2. b, Sample midplane images of PAR-2 in P0, P1 (dissected), P2 and P3 used for gradient measurements. c, Individual and average plots of PAR-2 distributions in P0, P1 (dissected), P2 and P3 cells, showing that the domain boundary interface occupies a proportionally larger fraction of the circumference in smaller cells. Note that full circumferential profiles around the entire cell are shown, normalized to cell circumference. Shaded regions highlight the interface regions between domains. The centre of the pPAR domain is at x = 0.1 and the centre of the aPAR domain at x = 0.5. d, Sample midplane images of PAR-2 at nuclear envelope breakdown in C27D9.1, wild-type or ima-3 P0 embryos, with arrowheads highlighting the boundary region. e, Plot of interface width versus embryo size for PAR-2 in C27D9.1 (n = 41), wild-type (n = 30) or ima-3 (n = 23) P0 embryos. f, g. The same as d, e but for PAR-6. Note that the interface width is effectively constant across a twofold size range. Sample sizes: C27D9.1 n = 56, wild type n = 20, ima-3 n = 36. Example fits shown in Supplementary Fig. 3. Scale bars, 10 µm.

Fig. 4 | Reaction kinetics and diffusion rates of PAR proteins fail to scale with cell size. Top row, PAR-6; bottom row, PAR-2. a, b, Cumulative step size distribution for PAR-6 (a) and PAR-2 (b) from all trajectories and embryos in e, d shown in comparison with a control membrane-associated molecule PH-PLCδ1. c, d, Plots of mean D versus cell size for PAR-6 (c) and PAR-2 (d) in wild-type (n = 6 and n = 9), ima-3 (n = 11 and n = 9) or C27D9.1 (n = 9 and n = 9) P0 embryos and C27D9.1 P1 embryos (n = 7 and n = 8). e, f, Plots of mean koff versus cell size for PAR-6 (e) and PAR-2 (f) in wild-type (n = 11 and n = 6), ima-3 (n = 3 and n = 4) or C27D9.1 (n = 6 and n = 5) P0 embryos. For c–f, linear regressions ± 95% confidence intervals are shown as solid lines plus shaded regions, respectively. g, h, Predicted size dependence of interface width D for PAR-6 (g) and PAR-2 (h) using the observed cell-size dependence of D and koff in a stochastic implementation of the PAR model. Mean ± s.d. shown as solid lines plus shaded regions, respectively; n = 20 simulations.
Off rates for varying cell sizes were measured using smPReSS (single-molecule photobleaching relaxation to steady state)\(^1\). In neither case did \(k_{\text{off}}\) scale with cell size. PAR-6 exhibited a modest correlation, with doubling of cell size leading to only a 50% decrease in \(k_{\text{off}}\) across the size range examined (Fig. 4c), and no correlation was observed for PAR-2 (Fig. 4f).

Reducing cell size disrupts polarity

We have so far shown that neither the patterns of PAR protein localization across the cell nor the reaction–diffusion kinetics that are thought to underlie these patterns exhibit scaling with cell size. In the context of our theoretical analysis, this general lack of scaling predicts the existence of a minimum size threshold for PAR polarity in the \(C.\) \(elegans\) \(P\) lineage.

To estimate the relevant size threshold (CPSS), we fitted a linear regression to experimental measurements of PAR protein kinetics and used this regression to specify \(D\) and \(k_{\text{off}}\) for PAR-2 and PAR-6 as a function of cell size (Fig. 4c–f). These rates were fed into a stochastic implementation of the two-component PAR model, which is similar to the PAR model above, but allows distinct behaviours of A and P molecules and integrates noise levels similar to those of experiments, allowing better comparison with in vivo data. Fitting the anterior and posterior PAR domain boundaries produced by this model resulted in similar values for \(\lambda\) as observed in vivo (Fig. 4g,h). Importantly, using the fit values for \(D\) and \(k_{\text{off}}\) we found no correlation between \(\lambda\) and cell size. Using these empirical measures of PAR protein kinetics, we obtained a predicted CPSS corresponding to a circumference of approximately 41 \(\mu\)m (Fig. 5d). Strikingly, this value roughly coincides with the size of P3 cells in wild-type embryos (41.5 ± 0.9 \(\mu\)m), which are the last of this lineage to divide asymmetrically. Thus, the diffusive behaviour of PAR proteins would be expected to impact the ability of cells to polarize at physiologically relevant length scales, potentially aiding the transition between asymmetric (P3) and symmetric (P4) modes of division.

To test these predictions, we turned to experimental reduction of embryo size. In this case, we examined the polarity of P3 cells in small \(ima\)-3 embryos relative to wild type and \(C27D9.1\). To quantify the polarity in P lineage cells, we applied selective plane imaging (SPIM) to embryos expressing PAR-2::GFP (green fluorescent protein) along with a membrane marker (Supplementary Video 3). This allowed us to generate a three-dimensional (3D) reconstruction of PAR-2 membrane distributions over time using image segmentation and identify the axis of maximal polarity. The axis of maximal polarity was defined as being perpendicular to a two-dimensional plane through the cell centre that maximizes PAR-2 intensity differences in the resulting two cell halves. Polarity was defined by \(1 - o_H\), where \(o_H\) is the overlap in histograms of PAR-2::GFP membrane intensities for the two cell halves, with reduced \(o_H\) reflecting increased asymmetry (Fig. 5a,b and Supplementary Table 1).

Wild-type P3 cells were 41.5 ± 0.9 \(\mu\)m in circumference, were distinctly polarized by 5 min before cytokinesis, and remained polarized throughout division (Fig. 5a,c–e). Their polarity was similar to
that of earlier P lineage cells (Fig. 5d; P0, P1, P2, P3 wild type). By contrast, P4 cells were 28 ± 0.7 μm with a reduced maximal polarity, consistent with the fact that these cells do not polarize and undergo asymmetric division (Fig. 5b–d). P3 and P4 cells from C27D9.1 embryos were similar in both size and polarity or lack thereof compared with wild type (Fig. 5c–e).

P3 cells from ima-3 embryos showed significant reduction in size to 35.2 ± 1.7 μm. At this size, P3 cells initially exhibited polarization comparable to that of the wild type (t = −5 min). However, as cells rounded up and approached cytokinesis, polarity declined, becoming indistinguishable from the polarity of P4 cells by 1 min before cytokinesis (Fig. 5c–e). To examine the consequences of this reduced PAR-2 polarity in P3 cells, we measured the resulting asymmetry of the P3 daughter cells—P4 and D. P3 daughter cells from ima-3 embryos showed reduced asymmetry in both cell size and PAR-2 levels (Fig. 5f–g). This loss of functional polarity in small P3 cells suggests that there is an in vivo size threshold between approximately 30 and 40 μm, below which PAR polarity is destabilized, thereby compromising division asymmetry, consistent with model predictions.

To provide further evidence that reduced size is the cause of symmetric P3 divisions in small embryos, we used laser-mediated extrusion to create mini-embryos, or mini-P0 cells (P0ex). Extrusion of posterior fragments of P0 early during polarity establishment yielded P0-like cell fragments that underwent a normal asymmetric P0-like division followed by an initially normal pattern of cell divisions35 (Fig. 6a,b and Supplementary Video 4). By contrast, P1-like cells (P1ex) were obtained by extrusion during late anaphase after the polarity of P0 was fully established (Fig. 6c,d). Importantly, P0ex cells were nearly as small as P1ex cells (Fig. 6g). Therefore, when P0ex cells divided to yield AB and P1 daughter cells, the resulting P1 daughter was significantly smaller than the P1ex cells. Thus, by allowing extruded cells to divide in vitro, we could assess the polarity and asymmetric division of the resulting differently sized P3 cells generated in these two conditions.

Extruded P0ex cells underwent the expected pattern of asymmetric divisions until the birth of P3, including the relative positions and timings of divisions, and yielded P0ex-derived P3 cells that were 28.8 ± 1.8 μm in circumference (Fig. 6b,c,g). However, these P3 cells exhibited symmetric divisions, showing reduced PAR-2 asymmetry before division and yielding two, similarly sized cells, with limited or no difference in PAR-2 inheritance. We denote these cells as P4* and D* on the basis of their positions. By contrast, P1ex-derived P3 cells were 38.1 ± 4.0 μm, exhibited polarized PAR-2 before division, and divided asymmetrically in all cases, with clearly asymmetric PAR-2 distributions and unequal cell sizes.
size (Fig. 6d,fg). Thus, reducing P3 size through either genetic or physical means resulted in loss of polarity and a premature switch from asymmetric to symmetric modes of division.

We conclude that the reaction–diffusion kinetics of the PAR proteins impose a minimal cell-size threshold for polarization. In failing to scale with cell size, this threshold can serve as a reference by which to facilitate cell-size-dependent switching from asymmetric to symmetric modes of division. We anticipate that similar processes may underlie fate switches in other asymmetrically dividing lineages, such as embryonic neuroblasts in Drosophila and stomatoid lineages in Arabidopsis, which undergo a limited number of self-renewing asymmetric divisions, with cell size decreasing with each division, ultimately culminating in a terminal symmetric division.53-55. The existence of a cell-size threshold in asymmetrically dividing lineages could help explain the tight control over not only fate but also size asymmetry at division, for example in both the C. elegans P lineage and Drosophila and C. elegans neuroblasts.56-60. Notably, loss of size asymmetry in Drosophila neuroblast divisions leads to premature decline in neuroblast size and reduced numbers of asymmetric neuroblast divisions61, consistent with a size-dependent loss of stem cell potential.

Cells tend to have defined sizes, which may be intimately connected to function, with changes in cell size linked to changes in fate.62 In many cases, fate choice affects cell size. Here we show the inverse, in which cell size limits fate choice. In this alternative paradigm, function follows form63-65: cells obtain information about their geometry through the impact of geometry on intracellular processes, which they can use to inform cell fate decisions, including when and how to divide.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41567-019-0601-x.

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Author contributions
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The authors declare no competing interests.

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Methods

Strains and reagents. Strain growth and media. C. elegans strains were maintained on nematode growth medium under standard conditions at 16 °C or 20°C unless otherwise indicated. Strains are listed in Supplementary Table 2.

RNAi. RNAi was performed according to described methods4. Briefly, HT115(DE3) bacterial feeding clones were inoculated from lysogeny broth (LB) agar plates to LB liquid cultures and grown overnight at 37 °C in the presence of 10 μg/ml carbenicillin. Bacterial cultures (100 μl) were spotted onto 60 mm agar RNAi plates (10μg/ml carbenicillin, 1 mM isopropyl-β-D-thiogalactoside) and L4 larvae were added to RNAi feeding plates and incubated for 20–48 h depending on gene and temperature. RNAi clones are listed in Supplementary Table 3.

Embryo dissection and mounting. For imaging, embryos were typically dissected in M9, egg buffer or Shelton's growth medium and mounted with 16–21 μm polystyrene beads (Polysciences) between a slide and coverslip or under a 2% agarose pad and sealed with a 1:1:1 mix of Vaseline, lanolin and paraflin wax (VALAP)5. 16–18 μm beads were used for single-molecule imaging to maximize the imaging surface. In most other cases, 21 μm beads were used to minimize compression effects on development. Dual-view inverted SPIM (diSPIM) imaging was performed in a water bath with the embryo mounted on a glass coverslip coated with a 2×2 mm² patch of poly-l-lysine (Sigma).

Microscopy and image acquisition. Confocal microscopy image acquisition. Midplane imaging was performed on a Nikon TiE with x63 or x100 objectives, further equipped with a custom X-Light V1 spinning-disc system (CrestOptics) with 50×, 488 nm, 561 nm fibre-coupled diode lasers (Obs) and an Evolve Delta (Photometrics). Imaging systems were run using MetaMorph (Molecular Devices) and configured by Cairn Research. For imaging of P lineage gradients in P2 to P4 in Fig. 3, 3D stacks were obtained and only embryos in which cells were almost parallel to the imaging plane were used for profile analysis.

Single-molecule image acquisition. Single-molecule imaging was performed as described in ref. 5. On a Nikon TiE with x100 numerical aperture 1.49 objective, further equipped with an iLas total internal reflection fluorescence (TIRF) unit (Roper), custom field stop, 488 nm, 561 nm fibre-coupled diode lasers (Obs) and an Evolve Delta (Photometrics). Imaging systems were run using MetaMorph (Molecular Devices) and configured by Cairn Research.

diSPIM image acquisition. SPIM images were acquired using a Marians LightSheet microscope (3i) with two ×40 numerical aperture 0.8 objectives. To minimize photobleaching, images were obtained with a single objective during an extended time lapse. Image stacks were typically acquired once per minute. The microscope system was run using SlideBook. To minimize potential pleiotropic effects on embryo development in small embryos, we standardized RNAi conditions to obtain small embryos that showed normal division patterns and cell arrangements, excluding excessively small embryos that had altered aspect ratios, which is known to affect development7. We also aimed, as far as possible, to score the relative timing and orientation of C, E and P lineage cells—Supplementary Table 3. In all cases where divisions and cell identities could be reliably scored, E divided before P in all but one case, suggesting that fate specification of P1 descendants is intact up to the P3 division.

Laser-mediated extrusion. For laser ablation and extrusion experiments, embryos were dissected and mounted in Shelton's growth medium. After inducing a hole in the eggshell using a 355 nm pulsed UV laser directed via an iLas Pulse unit (Roper), modest pressure was applied to the coverslip to extrude the relevant cell fragment. P1 extrusions were performed as the cleavage furrow was completing, P0 extrusions were performed around the time of symmetry breaking. Single image planes were captured at 1–2 min intervals to minimize phototoxicity.

Data analysis. Interface width. Interface width was measured from fluorescence intensity profiles extracted from midplane images of PAR-2 and PAR-6 in dual-labelled zygotes from nuclear envelope breakdown to the onset of cytokinesis, with two interface measurements obtained for each embryo (Supplementary Fig. 3). We observed a general sharpening of the interface beginning 60–100 s before furrow ingress for PAR-2 (Supplementary Fig. 3), which coincided with onset of cytokinetic ring assembly and a period of active alignment of PAR domain boundaries with the ingressing furrow. No sharpening was observed for PAR-6 (Supplementary Fig. 3).

The cortical profile was segmented at each time using the available fluorescent channels and custom-built software in MATLAB (MathWorks), and subsequently straightened in Fiji8, using a 20-pixel line thickness. Intensity profiles were obtained by averaging the brightest three pixels at each membrane position.

PAR-2 profiles were fitted using

\[ I(x) = \left( a + \frac{b}{2} \right) \left( \frac{\text{erf}(x - \epsilon) \sqrt{3}}{\lambda} \right) \]

where erf is the error function as implemented in MATLAB.

In a first round of fitting, the inflection point (interface centre) of the curve was determined. A second round of fitting was performed on a region of ±20 μm around the centre to determine λ. Fitting accuracy was then determined by smoothing the data using a Savitzky–Golay filter and subtracting the data from the fitting curve within the gradient region. If the maximum of the absolute difference exceeded an empirically chosen value (between 6% and 8% of the amplitude of the fitting function, depending on the noise level) the data were discarded. We averaged PAR-2 distributions at three consecutive time spaced 20 s apart approximately 3 min before furrow ingression, coinciding roughly with nuclear envelope breakdown. Among the three considered times at least two had to meet the threshold, otherwise the respective interface was not used for analysis.

PAR-6 profiles were initially fitted with an error function to determine their centre, top and ceiling. However, because the error function failed to capture the shape of the profile, the lower part of the curve was fitted with an exponential:

\[ I(x) = A e^{-c (x - \epsilon)} + c \]

using a 40% cutoff based on the top/bottom determined above to determine h. Varying the cutoff between 30% and 70% did not meaningfully alter the results, as expected for an exponential decay. Times for analysis were defined as for PAR-2.

When tracing the entire circumference of cells to obtain profiles, two gradient regions were obtained. When fitted individually, the two values of λ obtained for each embryo were not correlated (Supplementary Fig. 3) and hence each gradient region was treated as an independent sample.

Polarity of P cells from SPIM images. Polarity of P cells was assessed by first creating a 3D membrane rendering of PAR-2 fluorescence intensity obtained by diSPIM imaging, using custom-built MATLAB (MathWorks) software. Subsequently, the centre of mass was determined by averaging all positions of the membrane rendering. Next, a plane that cuts the centre of mass was rotated in all directions in steps of 5°, at each step dividing the cell into two halves. At each step the histogram of surface fluorescence intensity was determined on either side of the plane and the overlap of these (normalized) histograms was used as a measure of polarity. High overlap indicates that the two halves on either side of the bisecting plane are very similar, while no overlap indicates perfect polarity. The plane with minimal overlap (when the two sides are most different) is defined as the plane of maximum polarity.

Asymmetry for these cells is defined as 1—overlap and is what we report in Fig. 5.

Cell size. Cell size is typically reported as the circumference as measured directly from confocal microscopy images taken through the centre of the cell of interest. The only exception to this was for cell size calculated from 3D stacks taken by diSPIM. An effective circumference was calculated as that of a spherical cell of the same volume.

Asymmetry quantification. For size asymmetry measures of P3 daughters in Figs. 5f and 6g, cell-size measurements were made as above for the two P3 daughter cells and used to calculate an asymmetry index defined as

\[ \text{ASI} = \frac{|P_4 - D|}{|P_4 + D|} \]

with asymmetry reported relative to wild-type controls. For Fig. 6g, PAR-2 intensity was measured along the membrane of the daughter cells in a single midplane section, excluding the cell interface, subtracting chip background, and averaged. These values were then used to calculate the ASI as above, again normalized to wild-type controls. For Fig. 5g and Supplementary Table 1, membrane-associated GFP:PAR-2 was extracted as for SPIM analysis of P3 cell polarity above, and ω calculated to obtain a metric for asymmetry that was comparable to Fig. 5d.

Diffusion analysis. Tracking was performed in Python, using the trackpy package9, and custom code developed for our analysis (see code availability). Our analysis follows10. Briefly, mean squared displacement (MSD) was calculated for each particle and the first 10 lag times were fitted to MSD = 4Dr. For every embryo, a mean value for D was obtained by averaging D for all particles in the range 0.9 < c < 1.2. Notably, we used 20 ms exposures and 60 ms intervals between frames, as opposed to continuous imaging every 33 ms in ref. 10.

Off rate analysis—smPReSS. Dissociation rates were analysed as described in ref. 11 using the following fit equation for observed particle number N, assuming an infinite cytosolic pool:

\[ \frac{dN}{dt} = -k_{\text{on}}N + (k_{\text{off}} + k_{\text{on}})N \]

Here, k_{\text{off}} is the cytosolic rate on unbleached particles and k_{\text{on}} the bleaching rate induced by the imaging laser.

Modelling. Simplified two-component PAR system. The model used here was introduced in ref. 12 and a similar symmetric version was used in ref. 13. Briefly, the governing equations are
\[
\frac{\partial \phi}{\partial t} = D_A \frac{\partial^2 \phi}{\partial x^2} + k_{on} A_{cyt} - k_{off} A - k_{DP} PA \\
\frac{\partial \psi}{\partial t} = D_P \frac{\partial^2 \psi}{\partial x^2} + k_{on} P_{cyt} - k_{off} P - k_{PA} A^3 P \\
P_{cyt}(x,t) = p_0 - \psi P \\
A_{cyt}(x,t) = p_3 - \psi A
\]

where \(A\) and \(P\) denote membrane concentrations, \(A_{cyt}\) and \(P_{cyt}\) are (uniform) cytoplasmic concentrations, \(A\), \(P\) denote membrane averages, \(\psi\) is the system's surface-area-to-volume ratio, and \(p_0\) and \(p_3\) refer to the total amount of each protein species in the system. If not indicated otherwise, the following parameters were used: \(D_A = 0.1 \text{nm}^2\text{s}^{-1}\), \(k_{on} = 0.006 \text{nm}^2\text{s}^{-1}\), \(k_{off} = 0.005 \text{nm}^2\text{s}^{-1}\), \(k_{DP} = k_{PA} = 1 \text{nm}^2\text{s}^{-1}\), \(\psi = 30 \text{nm}^{-1} / \text{mm}^{-1}\) (half circumference) and a dosage ratio between \(A\) and \(P\) of 1:1. Surface-area-to-volume ratios were adjusted depending on cell size assuming a constant prolate-spheroid geometry (aspect ratio 27:15). All other parameters relating the cytoplasm and membrane were as described previously. To simplify analysis, note that this system is symmetric with the same values for diffusion and reaction rates for both PAR species. This assumption is reasonable, as empirical values for \(D\) and \(k_{on}\), the most relevant rates for gradient length, are similar for the two species. However, to calculate a realistic CPSS for comparison with experiments, we used the measured values for both species (Stochastic PAR system).

To assess qualitative behaviour of the PAR network on changing parameters, the governing system of partial differential equations was solved using an adaptive Runge–Kutta scheme\(^{43}\), using custom-built Python code (see code availability statement).

Simulations were initialized with two opposing domains with a sharp boundary and run until \(t = 10,000\) s. A simulation was said to break down within the time limit if the concentration of one species was larger than the other across the entire domain.

Wave pinning. The wave-pinning system was simulated using custom MATLAB (MathWorks) code, using the pdepe function, with parameters similar to the ones described previously\(^{44}\). For Figs. 1 and 2 parameters were changed and indicated in the figure legends with the following base set: \(\delta = 1/9\text{s}^{-1}\) (inactivation rate), \(\gamma = 1/9\text{s}^{-1}\) (maximal rate), \(D_{	ext{mem}} = 0.1\text{nm}^2\text{s}^{-1}\) (membrane diffusion), \(D_A = 100,000\text{nm}^2\text{s}^{-1}\) (cytoplasmic diffusion), \(L = 1\) (saturation parameter) and \(k_p = 0.067/9\text{s}^{-1}\) (basal conversion rate). Simulations were run until \(t = 10,000\) s. A simulation was said to have become unpolarized within the time limit if the difference between areas of high and low membrane concentration was less than 5%.

Mass-conserved activator substrate. The mass-conserved activator substrate model (OT) was implemented in MATLAB similar to Wave pinning, using Model I, previously described\(^{45}\), with the following parameters: \(D_A = 100,000\text{nm}^2\text{s}^{-1}\) (cytoplasmic diffusion), \(a_1 = 1\text{nm}^{-1}\), \(a_2 = 0.7\text{nm}^2\text{s}^{-1}\) and \(s = 1\), which approximates infinite diffusion. Here, \(a_i\) is a rate constant, \(a_i\) a concentration parameter and \(s\) sets the simulation. System size and membrane diffusion were chosen as indicated. Initial conditions for concentrations \(u\) and \(v\) were chosen as \(u(\text{t}=0, x) = c_{0u}(\text{x} - L/2)\) and \(v(\text{t}=0, x) = c_{0v}\), where \(c_{0u}\) and \(c_{0v}\) are plotted as \(A_{cyt}\) in Fig. 2a and \(\theta\) is the Heaviside step function. This sets the total amount of material due to mass conservation.

For the Goryachev model\(^{46}\) the following reaction terms were used, which have already been described elsewhere:\(^{47}\)

\[f(u,v) = a_1 uv^3 + a_2 uv - a_3 u\]

The following parameters were used to create the phase space diagram:

\(D_{	ext{mem}} = 0.1\text{nm}^2\text{s}^{-1}\), \(D_A = 100,000,000\text{nm}^2\text{s}^{-1}\), \(a_1 = 0.0067\text{nm}^2\text{s}^{-1}\), \(a_2 = 0.0033\text{nm}^2\text{s}^{-1}\), \(a_3 = 0.01\text{nm}^2\text{s}^{-1}\). The shape of initial conditions was the same as used for the Otsuji model above. Simulations were run until \(t = 10,000\) s. Polarity was scored the same as above for wave pinning.

Stochastic PAR system. Stochastic simulations of the PAR system were performed using a Gillespie algorithm\(^{48}\) implemented in MATLAB. The governing equations are

\[
\begin{align*}
\frac{\partial A}{\partial t} &= D_A \frac{\partial^2 A}{\partial x^2} + k_{on} A_{cyt} - k_{off} A - k_{DP} PA \\
\frac{\partial P}{\partial t} &= D_P \frac{\partial^2 P}{\partial x^2} + k_{on} P_{cyt} - k_{off} P - k_{PA} A^3 P \\
P_{cyt}(x,t) &= p_0 - \psi P \\
A_{cyt}(x,t) &= p_3 - \psi A
\end{align*}
\]

Note the different exponents conferring antagonism as well as different rate parameters for \(A\) and \(B\) compared with equation 1. Diffusion and dissociation rates were obtained from regressions in Fig. 4. Surface-area-to-volume ratios were dependent on cell size, assuming a prolate-spheroidal geometry with aspect ratio 27:15. All other parameter values were as described above (Simplified two-component PAR system) or as previously described. Breakdown of simulations at a given cell size was scored as described above for the deterministic system for averages of at least eight individual simulations.

Determining \(\lambda\) as a function of cell size and diffusion/reaction rates. To examine the dependence of \(\lambda\) on reaction and diffusion rates we chose \(L = 100\mu\text{m}\) to avoid strong boundary effects. All other rates were chosen as described in the respective figures and individual supplementary sections. Note that for Supplementary Fig. 1, because changing \(k_{on}\) alone alters membrane concentrations, to be able to vary \(\sqrt{k_{on}}\) across several orders of magnitude while still achieving a polarized state \(k_{on}\) had to be increased tenfold.

To explore how \(\lambda\) depended on system size, we kept the overall protein concentrations (per cell volume) constant and initiated the system with the same initial conditions as above. System size was varied using parameters as described for individual models.

For deterministic simulations, we determined the boundary length of simulated systems by measuring and inverting the maximum absolute slope of the concentration profile of membrane-associated species at steady state. To account for concentration differences across models and conditions, we normalized profiles to the maximum membrane concentration. For the stochastic model, interface profiles were fitted with an error function, using the same algorithm as for PAR-2 profiles, which facilitated direct comparison with experimental data.

Defining CPSS. To determine the CPSS for each system (Fig. 2a–c), we simulated across a parameter space grid defined by either total component concentrations (OT, GOR and WP) or relative component concentrations (PAR) and system size. On the basis of the criteria for each model stated above, this allowed us to define the polarized region of parameter space. CPSS was defined as the lowest simulated system size that permitted stable polarity domains. For the PAR model a biasing algorithm was used to refine the boundaries between regions, due to long simulation times.

Data availability
All data are included in the manuscript or Supplementary material.

Code availability
All model-related code is available at https://github.com/lhcgeneva/PARmodelling. Code for analysis and tracking of particle trajectories is available at https://github.com/lhcgeneva/SP1. Tracking was performed using the trackpy package (https://doi.org/10.5281/zenodo.60550).

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