Guiding self-organized pattern formation in cell polarity establishment

Peter Gross\textsuperscript{1,2,3,8}, K. Vijay Kumar\textsuperscript{3,4,8}, Nathan W. Goehring\textsuperscript{5,6}, Justin S. Bois\textsuperscript{7}, Carsten Hoege\textsuperscript{2}, Frank Jülicher\textsuperscript{3} and Stephan W. Grill\textsuperscript{1,2,3*}

Spontaneous pattern formation in Turing systems relies on feedback. But patterns in cells and tissues seldom form spontaneously—instead they are controlled by regulatory biochemical interactions that provide molecular guiding cues. The relationship between these guiding cues and feedback in controlled biological pattern formation remains unclear. Here, we explore this relationship during cell-polarity establishment in the one-cell-stage \textit{Caenorhabditis elegans} embryo. We quantify the strength of two feedback systems that operate during polarity establishment: feedback between polarity proteins and the actomyosin cortex, and mutual antagonism among polarity proteins. We characterize how these feedback systems are modulated by guiding cues from the centrosome, an organelle regulating cell cycle progression. By coupling a mass-conserved Turing-like reaction–diffusion system for polarity proteins to an active gel description of the actomyosin cortex, we reveal a transition point beyond which feedback ensures self-organized polarization, even when cues are removed. Notably, the system switches from a guide-dominated to a feedback-dominated regime well beyond this transition point, which ensures robustness. Together, these results reveal a general criterion for controlling biological pattern-forming systems: feedback remains subcritical to avoid unstable behaviour, and molecular guiding cues drive the system beyond a transition point for pattern formation.

Cell polarity establishment in \textit{Caenorhabditis elegans} zygotes is a prototypical example for cellular pattern formation that depends both on feedback between two classes of pattern-forming proteins and on upstream guiding cues provided by the centrosomal polarity trigger\textsuperscript{1–3}. Posterior PAR proteins (pPARs: PAR-1, PAR-2 and LGL-1) and anterior PAR proteins (aPARs: PAR-6, PAR-3 and PKC-3) interact via antagonistic feedback while bound to the cell cortex\textsuperscript{4}, which gives rise to stably unpolarized and polarized states\textsuperscript{5–7}. The formation of PAR polarity domains involves actomyosin cortical flows that transport PAR proteins\textsuperscript{8–11}. In addition, PAR proteins regulate the actomyosin cortex\textsuperscript{12–16}, implying mechanochemical feedback\textsuperscript{17}. Two centrosomal polarity triggers act as guiding cues for the polarization process\textsuperscript{1–3}. First, centrosomal microtubules protect PAR-2 from PAR-3-mediated antagonism. Second, contractility in the actomyosin cortex is increased via RhoA activation\textsuperscript{18}, and a local down-regulation of non-muscle myosin II (NMY-2, referred to as myosin) at the posterior pole\textsuperscript{19} initiates cortical flows\textsuperscript{20}. The relationship between guiding cues and feedback in PAR polarity establishment remains unclear. To address this issue, we first focus on feedback and later shift our attention to the guiding cues and their relation to the mechanisms of feedback.

Previous work has investigated antagonistic feedback between the two PAR species\textsuperscript{8,21}. We set out to characterize mechanochemical feedback from PAR proteins onto actomyosin contractility. Cortical myosin is regulated by PAR domains\textsuperscript{8,12–14}, but the strength of this regulation in terms of changes of myosin association and dissociation rates remains unknown. Fluorescence recovery after photobleaching (FRAP) is a technique for determining dissociation rates at steady state\textsuperscript{22,23} (Supplementary Fig. 1, Supplementary Section 1.2). Performing FRAP during maintenance is similar to the one reported during cortical flows, spontaneous dissociation rate of myosin in the anterior during maintenance is similar to the one reported during cortical flows, as measured via co-moving mass-balance imaging\textsuperscript{20} (COMBI). This indicates that myosin reaction kinetics do not change much over these stages of the cell cycle. We next determined the rate of NMY-2 dissociation from the cortex by considering that the steady-state surface concentration of NMY-2 is set by the ratio of this association rate and the dissociation rate from the cortex. To this end, we developed a fluorescence-based image quantification technique (MACE: membrane-associated concentration evaluation) that determines the spatiotemporal concentration fields of labelled proteins at the cell surface (Fig. 1a, see also ref.\textsuperscript{24}). We find that the rates of association of NMY-2 to the cortex are similar in both PAR domains (anterior: $k_{aa} = 0.19 \pm 0.03 \mu m^{-1}$; posterior: $k_{ap} = 0.21 \pm 0.03 \mu m^{-1}$) (Fig. 1d), indicating that NMY-2 association to the cortex is independent of PARs. However, changing the local PAR state of the cortex via RNAi changes the local NMY-2 dissociation rate (Fig. 1b, Supplementary Fig. 2k), indicating that the PAR domain state controls NMY-2 dissociation from the cortex. We next asked if the posterior or the anterior PAR complex regulates the NMY-2 dissociation rate. To this end, we measured the NMY-2 dissociation rate during \textit{par-2} and \textit{par-6} double-RNAi (Supplementary Fig. 2j). Under this condition, the NMY-2 dissociation rate is not much different from the one measured in the posterior domain of unperturbed embryos (Fig. 1b), indicating that the anterior PAR complex...
**Fig. 1 | Mechnochemical feedback in PAR polarity establishment.** a, Example of PAR and myosin protein concentration and velocity fields obtained at a single time point during polarity establishment from confocal medial sections using MACE (four minutes after flow onset; blue, PAR-2::GFP representing pPARs; red, PAR-6::mCherry representing aPARs; grey, NMY-2::GFP; see Methods). Scale bar, 5 μm. A and P denote the anterior and posterior, respectively. x indicates the position along the circumference, with the posterior pole at x = 0. b, Average NMY-2 dissociation rates as measured by FRAP for unperturbed, par-2, par-6 and par-2/par-6 double RNAi embryos as a function of position and cortical PAR state (see schematics at the bottom); see Supplementary Fig. 2d–g for statistics. c, NMY-2 dissociation rates as a function of PAR-6 concentration. Solid line represents a linear fit with slope $k_{AM} = -0.002±0.0007 \mu m^2 s^{-1}$ and intercept $k_{AM} = 0.117±0.009s^{-1}$. Due to uncertainties in the determining the PAR-6 concentration, condition (16) (par-2 RNAi, posterior side) was not included (Supplementary Fig. 2h). d, NMY-2 association rates as a function of position and cortical PAR state (see schematics at the bottom). Error bars are standard error of the mean.

...is the dominant regulator of the myosin dissociation rate\(^{36}\). Indeed, by combining FRAP data with MACE analysis we find that the dissociation rate $k_{AM}$ of NMY-2 decreases with increasing PAR-6 concentration approximately as $k_{AM} = (k_{AM} + k_{AM}<\text{PAR-6}>)$, where $k_{AM}$ is the spontaneous dissociation rate of NMY-2 in the absence of PARs. A is the anterior PAR-6 concentration and $k_{AM}$ is a coupling coefficient ($k_{AM} = (-2.0±0.7) \times 10^{-3} \mu m^2 s^{-1}$; Fig. 1c, Supplementary Fig. 2k). Thus, anterior PAR complexes control the amount of cortical myosin by regulating its dissociation rate. This gives rise to mechnochemical feedback since controlling myosin levels controls actomyosin flows, and thus flow-based PAR complex transport\(^{4,11,29}\).

Feedback structures allow non-equilibrium systems to spontaneously break symmetry and form self-organized patterns\(^{27-29}\). However, external signals can couple to self-organized Turing-like systems, for breaking symmetry and for guiding mechanisms of pattern formation to realize appropriate spatiotemporal profiles of constituents\(^{30-37}\). We next set out to investigate the relationship between feedback and guidance in PAR polarization, and develop a theory of guided mechnochemical self-organization where PAR distributions, myosin distribution and cortical flow pattern are all interdependent and respond to guiding cues. We aimed for a description that is simple enough to capture all relevant processes, but still contains enough detail to be of predictive power. Two feedback mechanisms are included: antagonism between anterior and posterior PAR proteins is captured by a mass-conserved Turing-like system\(^{38,39}\), and mechnochemical feedback is captured by coupling the Turing-like system to an active fluid to describe the mechanics of the actomyosin cortex\(^{26,30}\). In addition, two guiding cues modify PAR and myosin concentration fields over space and time to steer the polarization process. We write reaction–advection–diffusion equations for the cell surface concentration fields of PAR-2 (P), PAR-6 (A), and myosin (M) as representatives for the posterior PAR complex, the anterior PAR complex, and the contractile actomyosin cortex, respectively (Fig. 2a). We consider azimuthal symmetry around the long axis of the egg, and pursue a one-dimensional description with periodic boundary conditions, where the spatial coordinate x denotes the distance along the surface to the posterior pole ($-L/2 < x < L/2$, with L denoting the circumference of the embryo; Fig. 1a).

\[
\begin{align*}
\partial_t A &= -\partial_x (v A) + D_A \partial_x^2 A + \frac{k_{AM}}{1+k_{AM}P} A_{cyto} - k_{M,AM} A \\
\partial_t P &= -\partial_x (v P) + D_P \partial_x^2 P + k_{M,AM} A_{cyto} P - k_{M,AM} A P + c_P (x, t)
\end{align*}
\]
Fig. 2 | Isolating both guiding cues by RNAi and determining their spatiotemporal profiles. a, M, A and P represent myosin, aPARs and pPARs, respectively. These are located either in the cytoplasm or at the cortex, where they are subjected to lateral diffusion and advective transport by cortical flow. We consider spontaneous association and dissociation to and from the cortex, mutual inhibitory interactions between the aPARs and the pPARs in the cortex-associated state, as well as an aPAR-dependent regulation of cortical myosin concentration. Two polarity triggers steer the polarization process: one impacts on the cortical pPAR concentration (\(c_P\)), PAR-2-stabilization cue, see text for details), and the other impacts on the actomyosin cortex with two components (\(c_M\): myosin removal cue component, \(c_{\text{act}}\): contractility cue component, see text for details). b, Average spatiotemporal distributions of aPARs (PAR-6:mCherry) and pPARs (PAR-2::GFP) over the time-course of polarity establishment obtained by MACE and with mlc-4 RNAi to inhibit cortical flows (N=8 embryos, Supplementary Fig. 7b). c, d. Best fits from theory (displayed at t=50 s, 200 s and 450 s; solid lines; Supplementary Video 1) to MACE data (dots, shaded regions represent standard error of the mean) (c) are used to determine the spatiotemporal profile of the PAR-2 stabilization cue \(c_P\) (d), which reduces the anterior PAR complex-dependent inhibition of PAR-2. e, Average spatiotemporal profile of myosin (NMY-2::GFP) during par-2 and par-6 double RNAi to inactivate the PAR system (N=8 embryos, Supplementary Discussion and Supplementary Figs. 10 and 11). f, g. Corresponding average myosin flow field (N=10 embryos, see Methods). h, i, j. Best fits from theory (displayed at t=50 s, 200 s and 450 s; Supplementary Video 2) to MACE data (dots, shaded regions represent standard error of the mean) (h) are used to determine the spatiotemporal profile of the myosin removal cue component \(c_M\) (i), which increases the myosin dissociation rate, and the spatiotemporal profile of the actomyosin contractility cue component \(c_{\text{act}}\) (j), which controls the mechanical contractility of the cortex.

\[
\frac{\partial M}{\partial t} = -\nabla (vM) + D_A \frac{\partial^2 M}{\partial x^2} + D_M \frac{\partial^2 M}{\partial y^2} + k_{\text{on,MLC}}M - k_{\text{off,MLC}}M + k_{\text{act,MLC}}M + c_{\text{act}}(x,t) + c_M(x,t) + c_P(x,t)
\]

Here, \(v\) denotes the cortical flow velocity, and \(D_A\), \(D_M\) and \(D_M\) describe the respective diffusion coefficients of the three species in the cortex-associated state. Antagonistic feedback between the two PAR species is controlled by \(k_{\text{on,MLC}}\) and \(k_{\text{off,MLC}}\) (ref. [3]), and mechanical feedback arises from the regulation of the myosin dissociation rate by aPARs via \(k_{\text{act,MLC}}\) (Fig. 1c). Note that our experimental measurement of the PAR-6-dependent dissociation rate of myosin does not rule out additional regulatory interactions between the PAR proteins and the actomyosin cortex. Spontaneous binding and unbinding rates of \(S \in \{A, P, M\}\) are denoted by \(k_{\text{on}}\) and \(k_{\text{off}}\), respectively. Oligomerization states of aPAR and pPAR complexes are captured via effective coefficients, nonlinear interactions and the stoichiometric coefficients \(\alpha\), \(\beta\) (ref. [4]). All species obey mass conservation, and cytoplasmic concentrations \(A_{\text{cyt}}, P_{\text{cyt}}, A_{\text{cyt}}\) are determined by \(S_{\text{cyt}} = \rho_S - \rho_1 V \int_{L_1/L_2} S(x, t)\, dx\), where \(\rho_1 V\) is the total number of molecules of type \(S \in \{A, P, M\}\). Cytoplasmic volume is denoted by \(V\), and \(\psi\) is the surface-to-volume ratio. Finally, we consider two guiding cues, the PAR-2 stabilization cue \(c_P\) (reducing
ARTICLES

Fig. 3 | Predicting PAR and myosin dynamics in the presence of guiding cues and feedback structures. a–c, Average spatiotemporal distributions of aPARs (PAR-6::mCherry) and pPARs (PAR-2::GFP) (both N = 9 embryos) (a) and myosin (NMY-2::mKate2; N = 6 embryos) (b), together with the myosin flow field (N = 9 embryos) (c) during polarity establishment for the PAR-2 MT- mutant. d–f, Comparison of MACE data for the PAR-2 MT- mutant (dots, shaded regions represent standard error of the mean) to theory predictions (displayed at t = 50 s, 200 s and 450 s; see Supplementary Video 3). h–j, Average spatiotemporal distributions of aPARs (PAR-6::mCherry) and pPARs (PAR-2::GFP) (both N = 6 embryos) (h), myosin (NMY-2::GFP; N = 8 embryos) (i), together with the myosin flow field (N = 12 embryos) (j) during polarity establishment of the unperturbed condition. k–m, Comparison of MACE data for the unperturbed condition (dots, shaded regions represent standard error of the mean) to theory predictions (displayed at t = 50 s, 200 s and 450 s; solid lines); see Supplementary Video 4. g, n, Comparison of the measured and predicted PAR-2 domain width for the unperturbed condition (n) and the PAR-2 MT- mutant (g). Error bars are standard error of the mean.

The latter consists of two cue components, a myosin removal component \( c_{\text{M}} \) (increasing the spontaneous NMY-2 dissociation rate), and a contractility component \( c_{\text{M}}(t) \) (modifying overall contractility):

\[
\begin{align*}
  c_p(x, t) &= k_p A^\beta P(x)F_p(x)f_p(t) \\
  c_{\text{M}}(x, t) &= -k_{\text{off},\text{M}} M NMY-2(x)F_M(x)f_M(t), \quad c_{\text{M}}(t) = f_c(t)
\end{align*}
\]

Here \( F_p(x) \) and \( F_M(x) \) characterize the impact of the respective cues in a region around the posterior pole with a Gaussian profile, while \( f_p(t) \), \( f_M(t) \) and \( f_c(t) \) characterize the respective time-dependent behaviours of cues and cue components, smoothly transitioning between off- and on-states (Supplementary Equations (28)–(32), Supplementary Fig. 6). \( k_p \) and \( k_{\text{off},\text{M}} \) scale the amplitudes of the respective cues. Contractility \( C \) depends on myosin concentration \( M \), and gradients in \( C \) drive cortical flows, which are resisted by an effective cortex viscosity \( \eta \) and frictional drag \( \gamma \) with the membrane and cytoplasm according to:

\[
\frac{\varepsilon^2 \partial^2 C}{\gamma} - \frac{C}{\gamma} \partial_x C = \frac{C_{\text{M}}(t) M}{M + M_{\text{s}}}
\]

with \( \varepsilon = \sqrt{\eta/\gamma} \) a hydrodynamic length-scale, \( C_{\text{M}} \) a proportionality constant and \( M_{\text{s}} \) a contractility saturation constant. Supplementary Fig. 12 provides an analysis how sensitive this theory is to changes in each of the 28 parameters. Seven of these parameters are known (Supplementary Table 2). In this study, we directly measure ten more parameters (Supplementary Tables 2 and 3, Fig. 1c,d, Supplementary Figs. 3–6). We determine the remaining eleven parameters by a systematic parameter inference procedure, where numerical solutions are compared to the experimental dynamics of Fig. 2 (Supplementary Figs. 13–15, see Supplementary Section 2.6 for details). With this approach, we characterize spatiotemporal activity profiles of guiding cues, and calibrate our theory.
by determining all interactions and parameters of the PAR–actomyosin patterning system, as will be described below.

We first determined the spatiotemporal activity profiles of guiding cues, by fitting measured distributions of aPARs, pPARs and myosin together with the cortical flow to our theoretical description (Supplementary Section 2.6). For each cue we made use of RNAi to perform our analysis in a reduced system, where a guiding cue is singled out. First, we isolated the PAR-2 stabilization cue (c_p, Fig. 2a) by suppressing cortical flows via RNAi of the regulatory myosin light chain *mlc-4*, which inhibits advective transport of PAR proteins (Supplementary Fig. 7a,b). Using MACE, we showed that PAR domains still form under *mlc-4* RNAi (Fig. 2b), consistent with previous work. Here, the spatiotemporal dynamics of PAR proteins (Fig. 2b) are driven by the PAR-2 stabilization cue and PAR-reaction-chemistry-dependent feedback alone. Applying a systematic parameter inference procedure, we quantified four kinetic parameters of the PAR interaction network (the antagonistic interaction strengths \( k_{a\alpha}, k_{a\gamma} \) and the association rates \( k_{\text{on},A}, k_{\text{on},P} \); equations (1), (2) and (5), Supplementary Section 2.6, Supplementary Tables 1 and 2) together with the spatiotemporal activity profile of the PAR-2 stabilization cue onto the PAR system (Fig. 2d, Supplementary Sections 2.4–2.6).

With this, we can quantitatively account for the measured PAR distributions over the entire polarization process under *mlc-4* RNAi (Fig. 2c, Supplementary Video 1). We find that the PAR-2 stabilization cue is turned on with a characteristic timescale of 74 ± 4 s, acts over almost half the surface of the embryo (width: 57.2 ± 0.3 μm) and protects approximately 95% of the PAR-2 proteins from aPAR-dependent antagonism near the posterior pole (Fig. 2d, Supplementary Table 2). To conclude, we have quantified the spatiotemporal activity profile of the PAR-2 stabilization cue.

Next, we characterized the actomyosin guiding cue, which triggers cortical flows. This cue consists of two components: first, a contractility component initially increases myosin activity throughout the entire cortex in preparation for polarity establishment, and later globally down-regulates myosin activity when PAR domains are established. Second, a myosin removal component locally removes myosin in the vicinity of the centrosome at the posterior pole for a transient period of time, to generate a contractile imbalance that initiates cortical flows. Since both the PAR system (Fig. 1c) and this two-component actomyosin guiding cue impact on myosin, we set out to render the PAR system ineffective in order to isolate the actomyosin cue.

Double-RNAi of *par-2* and *par-6* reduced the PAR-2 and PAR-6 amounts to a degree such that the regulation of PARs on myosin is negligible (see Supplementary Section 1.1 and Supplementary Figs. 10 and 11). An active-fluid description of actomyosin (equations (3), (5) and (6); Supplementary Table 1) together with a systematic parameter inference procedure (Supplementary Sections 2.4–2.6) allowed us to quantify two physical parameters of the actomyosin system (\( C_\text{c} \) and \( M_\alpha \), which control the relation between myosin concentrations and contractility, Supplementary Section 2.6, Supplementary Tables 1 and 2) together with the activity profile of both components of the actomyosin cue. With this, our theory describes at a quantitative level the measured NMY-2 distribution and cortical flow field under double RNAi of *par-2* and *par-6* (Fig. 2f,h, Supplementary Video 2). We find that the myosin removal component of the actomyosin cue down-regulates NMY-2 by increasing its dissociation rate by a factor of about six in a restricted region close to the posterior pole (width: 31.7 ± 1.2 μm) for a time of 204 ± 10 s (Fig. 2i). The contractility component of the actomyosin cue is active even before polarity initiation at \( t = 0 \) s, and its time of inactivation appears to coincide with the inactivation of the myosin removal component (Fig. 2i, Supplementary Fig. 5). To summarize, we have inactivated mechanochemical feedback to isolate both the PAR and the actomyosin subsystems to determine the strength of all unknown interactions in our theoretical description and have quantified the spatiotemporal profile of both guiding cues.

We next tested if our theory correctly describes how feedback structures and guiding cues together orchestrate PAR polarity.
establishment. To this end, we set out to predict the spatiotemporal evolution of PAR polarization in the full system and in the presence of mecanochemical feedback (Fig. 1c), starting from the unpolarized state that is perturbed by both cues (Fig. 2). We investigated two conditions: unperturbed PAR polarity establishment (Fig. 3h–i) as well as PAR polarity establishment for a PAR-2 mutant with an inactive PAR-2 stabilization cue (referred to as PAR-2 MT--; Fig. 3a–c). We obtained a theoretical prediction for both conditions by numerically solving equations (1)–(6) (Supplementary Table 1) using above-determined parameter values (Supplementary Tables 2 and 3) and setting the strength of the PAR-2 stabilization cue to zero for the PAR-2 MT- condition. We find good agreement between theoretical predictions and experimental data for all fields measured (Fig. 3d–g,k–n, Supplementary Videos 3 and 4). PAR-2 dynamics are captured better for PAR-2 MT- as compared with the unperturbed case, which is indicative of an alteration of the spatiotemporal dynamics of the PAR-2 stabilization cue between the unperturbed and the mlc-4 RNAi condition we have not considered (Supplementary Fig. 9, Supplementary Video 5). Hence, we are able to account for the spatiotemporal dynamics of the full PAR–actomyosin system, and we conclude that our theory appropriately captures both guidance and feedback.

We next set out to investigate the relationship between feedback and guidance. For this, we studied the temporal evolution of the full system in terms of the total numbers of proteins of PAR-2, PAR-6 and NMY-2 associated with the cortex, respectively. We set out to reveal in our theory which interactions drive the system from the unpolarized to the polarized state, differentiating between terms associated with guidance and terms associated with feedback (see Methods; Fig. 2a). We make two observations: first, instantaneously setting to zero all terms associated with guidance prior to approximately 60 s results in the system returning back to the initial and unpolarized state, whereas the system continues to progress towards the polarized state when these terms are set to zero beyond this point in time (Supplementary Fig. 8). Second, the contribution of guidance terms to the time evolution of the trajectory switches from assisting to impeding (Fig. 4a, light blue arrowheads; also see Methods) at about 260 s. Together, this reveals three phases of polarity establishment: guiding cues are required in the initial phase up to 60 s (transition point, Fig. 4a,e, Supplementary Fig. 8), guiding cues are no longer required but assist the polarization process in a second phase up to 260 s (guidance release, Fig. 4a,e), and guiding cues impede the process and self-organization takes over in determining the systems’ dynamics in the final phase (Fig. 4a,e).

Fig. 5 | Robust PAR polarization breaks down close to the transition point. a, Two individual PAR-2 MT- mutant embryos that were exposed to different mlc-4 RNAi feeding times, resulting in reduced peak cortical flow velocities \( v \) (Supplementary Fig. 7c). Left: no PAR domains. Right: PAR domains form. Scale bar, 5 μm. b, Cortical flow velocities for 28 zygotes, exposed to weaker-to-stronger mlc-4 RNAi. Upper row and yellow circles, embryos successfully polarized \( (N=15) \); bottom row and grey diamonds, embryos failed to polarize \( (N=13) \). Green vertical line indicates the theoretically predicted critical velocity \( v_{\text{crit}} \) required for polarization by the actomyosin cue \( (c_{\text{M}}) \). The peak cortical flow velocity of unperturbed yzogotes (mean ± SEM) is also indicated. c, Inhibition of the centrosomal polarity trigger via laser ablation. Both centrosomes were ablated, while \( t=0 \) s represents the ablation of the second and final centrosome. Dependent on the time-point of ablation, the posterior PAR domain formed (upper row) or failed to form (lower row). Scale bar, 5 μm. d, The PAR-2 domain at the moment of the final centrosome ablation. Green line: theoretically predicted transition state. Yellow: yzogotes that formed a posterior PAR domain after centrosome ablation \( (N=7) \); grey: yzogotes that did not form a domain \( (N=5) \). Inset: Integrated number of cortical PAR-2 for all yzogotes from d. Yellow, right column: posterior PAR domain formed \( (N=7) \), grey, left column: posterior PAR domain failed to form \( (N=5) \), green: transition state. Errors represent the standard deviation and were determined from the uncertainty in the fluorescence calibration factor.
Our analysis indicates that two features are important for achieving controllability and robustness. First, feedback remains subcritical throughout to avoid unstable behaviour and spontaneous pattern formation. Consistent with this, C. elegans zygotes have not been observed to polarize in the absence of cues\(^{24,44,46}\), but how far the PAR–actomyosin patterning system is placed away from a mechaenochemical instability is not known\(^{14,48}\). We tested for subcritical feedback in our theory by performing a linear stability analysis of the homogeneous state. We find that the system is poised far away from the unstable regime (Fig. 4b, white region), which allows for control of pattern formation since the system will polarize only in response to guiding cues. Second, to achieve robustness, guiding cues need to be strong enough and active long enough to drive the system significantly beyond the ‘barrier to polarization’ (that is, the transition point at 60 s, Fig. 4a). For example, our theoretical analysis suggests that the actomyosin guiding cue is not strong enough to drive the system beyond the transition state when contractility drops below a critical threshold (Fig. 4b, grey region), and predicts a critical flow speed of 3.7 μm min\(^{-1}\) required for polarization (Fig. 5b). To test this, we isolated the actomyosin cue (PAR-2 MT\(^{-}\) mutant condition, Fig. 5a) and gradually reduced cortical flow speeds by performing a series of milder-to-stronger \(\pm 1\) RNAi experiments. Consistent with our prediction, embryos failed to polarize when cortical flow speeds dropped to about half the flow speed of unperturbed embryos (6.9 ± 0.3 μm min\(^{-1}\); Fig. 5b). Hence, the actomyosin cue generates cortical flows that are approximately twice the critical speed required for successful polarization, which provides robustness. Next, we experimentally investigated how long the PAR-2 stabilization cue needs to be active to achieve polarization. We laser-ablated centrosomes at different times (ref. 14, Fig. 5c), recorded the spatial profiles of the nucleated PAR-2 domain at the moment of ablation for each experiment, and assessed if embryos continued to evolve towards the polarized state afterwards, or returned back to the unpolarized state (Fig. 5c, see Methods). We found that the minimal PAR-2 domain required for polarization in the experiment (Fig. 5d) was similar to the PAR-2 domain at the transition point as predicted from theory (Figs. 4a and 5d). Notably, although approximately 70,000 PAR-2 proteins are cortex-associated at the time of guidance release (\(t = 260 s\), Fig. 4a), only about 10,000 PAR-2 proteins are required to reach the transition point that needs to be passed for achieving polarization (Fig. 5d). Hence, the PAR-2 stabilization cues drives the system far beyond the ‘barrier to polarization’, which provides robustness.

A fundamental challenge for patterning systems is that they typically involve unstable behaviours that are hard to control. Our analysis indicates that polarity pattern formation in C. elegans avoids local instabilities. Feedback structures are subcritical but generate basins of attraction around (at least) two states, one corresponding to the unpatterned and one to the patterned state (Fig. 4c). Guiding cues drive the system away from the unpatterned state (Fig. 4d) and beyond its basin of attraction, and into the vicinity of the patterned state. Finally, self-organization can take over in shaping the time evolution of the system near the patterned state (Fig. 4e). Thus, guiding cues are active for a significant fraction of the trajectory, rendering PAR polarization deterministic and probably conferring robustness. Note that guiding cues can be either temporary and functioning only in triggering pattern formation (as is the case for the actomyosin cue, Fig. 2h;1j) or permanent and with a possible role in shaping the final patterned state (PAR-2 stabilization cue, Fig. 2d). Interestingly, two guiding cues shape the self-organized dynamics of the system, and these dynamics are generated by two distinct feedback structures (PAR antagonism, mechaenochemical feedback). We speculate that combining several feedback structures and controlling each one with its own guiding cue is a general mechanism for providing specificity and robustness in controlled biological pattern formation.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at [https://doi.org/10.1038/s41567-018-0358-7](https://doi.org/10.1038/s41567-018-0358-7).

Received: 17 July 2018; Accepted: 23 October 2018; Published online: 03 December 2018

### References


Acknowledgements
PG. acknowledges a EMBO Long-Term Fellowship for funding. The research of K.V.K. is supported by the Department of Biotechnology, India through a Ramalingamswami Re-entry Fellowship, and the Max Planck Society and the Department of Science and Technology, India through a Max Planck Partner Group at ICTS-TIFR. N.W.G. was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001086), the UK Medical Research Council (FC001086) and the Wellcome Trust (FC001086), and is a member of the GENIE network supported by COST Action BMI 408 and EMBO. S.W.G was supported by the DFG (SPP 1782, GSC 97, GR 3271/2, GR 3271/3, GR 3271/4), the European Research Council (grants 281903 and 742712), ITN grants 281903 and 641639 from the EU, the Max-Planck-Society as a Max-Planck-Fellow, and the Human Frontier Science Program (RGP0023/2014). J.S.B acknowledges the Human Frontier Science Program for funding. We thank D. Dickinson, B. Goldstein, F. Motegi and G. Seydoux for sharing C. elegans strains. We thank P. Gönczy, L. Hubatsch, T. Hyman, K. Kruse and M. Labouesse for discussion and insightful comments on the manuscript.

Author contributions
PG. performed experiments and K.V.K. developed the theory, with help from all authors. Data were analysed together with input from all authors. P.G., K.V.K., F.J. and S.W.G. wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41567-018-0358-7. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.W.G.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2018
Methods

C. elegans strains and growth conditions. All strains were maintained at 18°C and imaged at room temperature. Before imaging, C. elegans was transferred to 24°C for 12–16 h. To obtain embryos, adult worms were dissected in M9 buffer (22 mM KH2PO4, 42 mM Na2HPO4, 86 mM NaCl) and mounted on agarose pads for imaging. All C. elegans strains used in this study are described in Supplementary Table 4. RNA interference was performed using the feeding method, as described in ref. 20. Feeding times were standardized for each condition, and were typically between 16 h to 24 h.

Image acquisition. All spinning-disk confocal movies of C. elegans zygotes were, except otherwise noted, acquired at room temperature, using a Zeiss Axiovert Observer Z1 equipped with a CSU-X1 Yokogawa spinning disk head using a 63X/1.2 NA PlanApochromat objective and a Andor iXon emCCD camera. Measurements of the threshold velocity for polarity establishment were performed using the same system equipped with a Hamamatsu ORCA-Flash4.0 V2 CMOS camera. Concentration measurements were performed by acquiring one confocal stack in the mid-plane of the C. elegans zygote with a frame rate of 10 s, to reduce photobleaching to below 5% for 500 s. Given a typical number of frames per movie of about 70, we estimate the effect of photobleaching to about 5% over the course of our experiments (Supplementary Fig. 3d). NMY-2 GFP movies used for flow measurements were obtained in the mid-plane with a frame rate of 1 s, whereas NMY-2 mKate movies were captured with a frame rate of 2 s. Fluorescent correlation spectroscopy measurements were acquired with a Zeiss LSM 780 confocal microscope.

FRAP on NMY-2. We performed the FRAP experiments using an Andor FRAPPA system. We acquired the images as cortical Z-stacks of three planes at 0.5 μm spacing with an interval time of 2 s between individual stacks (iQ software, Andor Technology). We performed FRAP on GFP-labeled NMY-2 by bleaching a square of (9 μm × 9 μm) with a laser dwell time of 20 μs per pixel. We monitored the recovery for a subsquare (6 μm × 6 μm) at the centre of the bleach square. We fitted the recovery curves of each FRAP experiment and determined the characteristic recovery time by fitting the fluorescence-intensity data with an exponential function, from which the mean as well as the standard error of the mean was calculated (Supplementary Fig. 2d–g). In case that the protein reached a steady-state concentration before the FRAP event, this recovery timescale is identical to the dissociation timescale of the bleached protein (Supplementary Discussion 1.2, Supplementary Fig. 1).

Membrane-associated concentration evaluation (MACE). The fluorescence intensity for GFP, mCherry and mKate2 was calibrated as follows. Initially, we measured the fluorescence intensity of these solutions with known fluorophore concentration and mKate2, using a Varian Cary 4000 UV-Vis Spectrophotometer. We then calculated (Supplementary Fig. 2d–g). In case that the protein reached a steady-state concentration before the FRAP event, this recovery timescale is identical to the dissociation timescale of the bleached protein (Supplementary Discussion 1.2, Supplementary Fig. 1). Membrane-associated concentration evaluation (MACE). The fluorescence intensity for GFP, mCherry and mKate2 was calibrated as follows. Initially, we measured the fluorescence intensity of these solutions with known fluorophore concentration and mKate2, using a Varian Cary 4000 UV-Vis Spectrophotometer. We then calculated (Supplementary Fig. 2d–g). In case that the protein reached a steady-state concentration before the FRAP event, this recovery timescale is identical to the dissociation timescale of the bleached protein (Supplementary Discussion 1.2, Supplementary Fig. 1).

Since \( \sigma \) characterizes the length where the amplitude drops by \( e^{-1} \), the PSF volume is characterized by an ellipsoidal shape

\[
\frac{x^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2} + \frac{z^2}{\sigma_z^2} = 1
\]

with a volume of

\[
V_{PSF} = \frac{4}{3} \pi \sigma_x \sigma_y \sigma_z
\]

and an illuminated area of a membrane in the xz plane

\[
A_{PSF} = \pi \sigma_x \sigma_z
\]

This implies that the surface concentration is given by:

\[
\epsilon = \frac{N}{A_{PSF}} = \frac{(\rho) V_{PSF}}{A_{PSF}} = \frac{V_{PSF}}{w A_{PSF}} = \frac{4}{3} \pi \rho w^{-1}
\]

Here, \( \sigma_x \) denotes the surface concentration, \( N \) the number of proteins in the confocal volume, \( A_{PSF} \) the surface of the membrane illuminated by the confocal microscope, \( V_{PSF} \) the volume of the point spread function, \( w \) the width of the point spread function in the y direction, \( \rho \) the fluorophore density, \( w \) the fluorescence calibration factor (Supplementary Fig. 3c) and \( I \) the intensity. Note that we choose the coordinate system such that \( x \) and \( z \) lie in the membrane plane and \( y \) is perpendicular to the membrane. The fluorescence intensity \( I \) was obtained, for each individual profile along the membrane, by fitting the intensity profile with:

\[
I(x) = \frac{I}{\sigma^2} e^{-\frac{(x-x_0)^2}{2 \sigma^2}} + I_{	ext{ramp}} \left/ 2 (I_{	ext{ramp}} (x-x_0) + 1) \right.
\]

We binned these resulting kymographs over 2.17 μm in space (10 pixel).

Generating ensemble-averaged concentration and flow fields. We synchronized individual kymographs in space and time using the geometric posterior pole and the beginning of cortical flows as reference points (\( x = 0, t = 0 \)). For PAR concentration fields for mKate protein, we synchronized individual kymographs
in time to the onset of cytokinesis. We then characterized the time between polarity establishment and cytokinesis to typically 850 s, and then generated ensemble-averaged PAR concentration fields where \( t = 0 \) denotes triggering of polarity establishment. For creating ensemble-averaged concentration fields, we discarded all measurements that show non-stereotypic initiation of cortical flows, away from the posterior pole (with a tolerance of 10 \( \mu \text{m} \)).

**Determining total protein amounts.** We determine the total protein amount as the sum of the cortical and the cytoplasmic amount. The cytoplasmic amount was determined by determining the average cytoplasmic concentration, as explained above, and integration over the ellipsoidal yolk, using \( a = 27 \mu \text{m}, b = 15 \mu \text{m} \) and \( c = 15 \mu \text{m} \) as semi-axes of the ellipsoid. The cortical amount was determined by first generating calibrated kymographs of the protein of interest. We then integrated the protein amount over the ellipsoid. Therefore, we first reparameterized the kymographs from distance to the posterior to angle with respect to the anterior-posterior axis. Simplifying the embryonic shape as ellipsoidal, we can then integrate the protein amount using:

\[
P(P_{\text{cell}}) = \int_{0}^{2\pi} \int_{0}^{\pi/2} (b^2 c^2 \sin(\phi) \cos(\theta))
\]

with \( P_{\text{cell}} \) the protein amount on the cell surface and \( P(\phi, \theta) \) as the protein concentration on the cell surface. Assuming azimuthal symmetry, a kymograph along \( \Theta \) provides sufficient information on the total cortical bound protein fraction (Supplementary Fig. 3i–q).

**FCS measurements of the total protein amounts.** We first determined the confocal volume of the Zeiss LSM 780 confocal microscope. To accomplish this, we measured autocorrelation spectra for GFP in solution, with four different concentrations (300 nM, 100 nM, 10 nM, 3 nM). Fitting each autocorrelation allows for the determination of the number of fluorophores in the confocal volume, which translates to a confocal volume in the case that the fluorophore concentration is known \( \gamma \), yielding \( V_{\text{conf}} = 3.2994 \times 10^{-13} \text{m}^3 \). Next we determined the number of PAR-2::GFP molecules inside the confocal volume, in the one-cell embryo by measuring autocorrelation spectra, for six embryos at a total of 21 time points. We then characterized the time between the appearance of the centrosome, which occurs in case the centrosome was not re-appearance of the centrosome, which occurs in case the centrosome was re-activated, by calculating the time it takes for a given value of PAR-2 to reach a plateau value. The error in the PAR-2 concentration was determined at each time point by fitting the function \( \text{erf}((\mu - x)/s) \) to unlabelled protein by western blotting (Supplementary Equations (22)–(24)) and guidance interactions (Supplementary Equations (25) and (26)). These interactions give rise, at each time point, to a change in the number of cortical PAR-2, PAR-6 and NMY-2. For our quantification of the PAR-3 and PAR-6 amount, we used strains that possess a fluorescently tagged PAR-2 and PAR-6 (using the SWG025 strain) in the confocal mid-plane of the zygote. These embryos were exposed to a range of different feeding times of mlc-4 RNAi. We determined cortical flow velocities for mlc-4 RNAi by investigating the displacement field of yolk granules adjacent to the actomyosin cortex, using the freely available PiLab MATLAB algorithm \( \gamma \). Two-dimensional velocity fields were obtained as in ref. \( \gamma \) (Supplementary Fig. 7c). Each embryo then was classified as polarized or unpolarized by monitoring the occurrence of a posterior PAR domain close to cytokinesis and an asymmetric cell division. We determined the theoretical prediction by calculating the trajectory of the PAR-actomyosin system. Notably, we observed that initially the cues drive the system towards the patterned state. If the cues are however active for a longer time, the system can cross the transition point, from which point on the self-organized interactions will carry the system to the patterned state, even when the cues are inactivated (Supplementary Fig. 8a).

**Separating guidance from self-organized components during trajectory analysis.** In Supplementary Equations (33)–(35), we separated interactions that arise to PAR polarity establishment into self-organized and guiding components (Supplementary Equations (22)–(24)) and guidance interactions (Supplementary Equations (25) and (26)). These interactions give rise, at each time point, to a change in the number of cortical PAR-2, PAR-6 and NMY-2. For our quantification of the PAR-3 and PAR-6 amount, we used strains that possess a fluorescently tagged PAR-2 and PAR-6 (using the SWG025 strain) in the confocal mid-plane of the zygote. These embryos were exposed to a range of different feeding times of mlc-4 RNAi. We determined cortical flow velocities for mlc-4 RNAi by investigating the displacement field of yolk granules adjacent to the actomyosin cortex, using the freely available PiLab MATLAB algorithm \( \gamma \). Two-dimensional velocity fields were obtained as in ref. \( \gamma \) (Supplementary Fig. 7c). Each embryo then was classified as polarized or unpolarized by monitoring the occurrence of a posterior PAR domain close to cytokinesis and an asymmetric cell division. We determined the theoretical prediction by calculating the trajectory of the PAR-actomyosin system. Notably, we observed that initially the cues drive the system to the patterned state. If the cues are however active for a longer time, the system can cross the transition point, from which point on the self-organized interactions will carry the system to the patterned state, even when the cues are inactivated (Supplementary Fig. 8a).

**Determining the ratio of labelled to unlabelled protein.** For our quantification of the PAR-2 and PAR-6 amount, we used strains that possess a fluorescently tagged transgene and the endogenous, untagged gene. We quantified the ratio of labelled to unlabelled protein by western blotting C. elegans embryos against PAR-2 and PAR-6 (Supplementary Fig. 3f). We then determined the theoretical prediction by calculating the trajectory of the PAR-actomyosin system. Notably, we observed that initially the cues drive the system to the patterned state. If the cues are however active for a longer time, the system can cross the transition point, from which point on the self-organized interactions will carry the system to the patterned state, even when the cues are inactivated (Supplementary Fig. 8a).

**Determining the critical flow velocity for polarization via flows.** To measure the threshold velocity for polarity establishment, we determined the fluorescein-stained PAR-2 and PAR-6 (using the SWG025 strain) in the confocal mid-plane of the zygote. These embryos were exposed to a range of different feeding times of mlc-4 RNAi. We determined cortical flow velocities for mlc-4 RNAi by investigating the displacement field of yolk granules adjacent to the actomyosin cortex, using the freely available PiLab MATLAB algorithm \( \gamma \). Two-dimensional velocity fields were obtained as in ref. \( \gamma \) (Supplementary Fig. 7c). Each embryo then was classified as polarized or unpolarized by monitoring the occurrence of a posterior PAR domain close to cytokinesis and an asymmetric cell division. We determined the theoretical prediction by calculating the trajectory of the PAR-actomyosin system. Notably, we observed that initially the cues drive the system to the patterned state. If the cues are however active for a longer time, the system can cross the transition point, from which point on the self-organized interactions will carry the system to the patterned state, even when the cues are inactivated (Supplementary Fig. 8a).

**Determining the transition-state PAR-2 domain size by centrosome laser ablation.** Laser ablation of the centrosome was performed in a C. elegans strain with 3DF-labeled with GFP and PAR-2 labeled with mNeonGreen, while using mlc-4 RNAi. We conducted centrosome ablation using methods similar to those in ref. \( \gamma \). We performed ablation by applying 10 ultraviolet pulses at 1 kHz on the circumference of a circle with a diameter of 0.5 \( \mu \text{m} \) at 9 equidistant sites. We ablated both centrosomes at different time points during the first cell stage, when their distance to the membrane was larger than typically 2 \( \mu \text{m} \), to avoid membrane rupture. After ablation, we tagged the zygote for typically 5 min. We scanned for the re-appearance of the centrosome, which occurs in case the centrosome was only bleached, for cell rupture or for membrane damage, resulting in cytoplasmic leakage, and excluded all these from our dataset. From 72 centrosome ablation experiments, 12 were considered successful. At the time point of the second centrosome ablation, we used MACe to measure the spatial profile of the PAR-2 domain as well as the integrated number of cortical PAR-2. The error in the PAR-2 concentration was determined by the standard deviation in the calibration factor.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Code availability.** Code to analyse the data and perform numeric simulations is available upon request.
Data availability
All data generated or analysed in this study are available from the corresponding author upon request.

References