Supplementary Information for

Modelling protein dynamics in *C. elegans* embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms

Sofia Barbieri¹*, Aparna Nurni Ravi ², Erik E. Griffin ², Monica Gotta¹*

¹ Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Geneva 1211, Switzerland.
² Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

*Sofia Barbieri
E-mail: sofia.barbieri@unige.ch

*Monica Gotta
E-mail: monica.gotta@unige.ch

This PDF file includes:

Supplementary text
S1 references
Figures S1 to S5
Tables S1 to S7
Legends for Supplementary Movies S1 to S3

Other supplementary materials for this manuscript include the following:

Supplementary Movies S1 to S3
Supplementary Information Text

1. Supplementary Methods

Strains and maintenance

The C. elegans strains used in this work were knock-in CRISPR strains where PLK-1 and MEX-5 were tagged with mCherry at the N-terminus (see Movie S1A-B). The mCherry::mex-5 (JH3296) is a gift of Prof. Geraldine Seydoux, the plk-1::sgfp (OD2425) is a gift of Dhanya Cheerambathur (1). The strain plk-1(syb2724) (PHX2724, in the paper referred to as mCh::plk-1) was designed and generated by SunyBiotech. The sequence inserted at the N-terminus was: mCherry-ENLYFQSGKGAGS to as mCh::plk.

For Single colonies of RNA interference 13.5%. For all the experiments, N=30.

The strain mCh::plk-1; par-1(zu310) (KK822 (2)), with PHX2724 males. All strains were cultured in standard conditions at 20°C, except for the par-1 temperature sensitive strains was measured for FRAP experiments in Fig. 2A and 2B, mCh::plk-1 and mCh::mex-5 worms were shifted from 20°C to 25°C overnight before imaging.

Embryonic lethality

mCh::mex-5, mCh::plk-1 and of the plk-1::sgfp worms were singled in small NGM plates with OP50 bacteria at the L4 stage. They were left to lay eggs for 24 h at 20°C, then the adults were removed from the plate. Eggs were left to develop and hatch for 24 h 20°C. The number of unhatched eggs was counted with respect to the total progeny (dead eggs and larvae), the lethality was calculated as their ratio and was as followed: mCh::mex-5 embryos 0.9 ± 1.5%, mCh::plk-1 embryos 11.6 ± 7.9%, plk-1::sgfp embryos 1.1 ± 1.7%. Lethality of the par-1 temperature sensitive strains was measured at the permissive temperature of 15°C and was: par-1(zu310) embryos 0%, mCh::plk-1; par-1(zu310) embryos 22.4 ± 13.5%. For the all experiments, N=30.

RNA interference

Single colonies of HT115 bacteria containing L4440 or T444T empty vectors (control conditions, labelled “ctrl(RNAi)” in the figures), and HT115 bacteria with plasmids for m5-5(RNAi) (3), mbk-2(RNAi) (4) and m5-5(RNAi) (5) were inoculated in LB medium + carbenicillin (1 mM).

For m5-56(RNAi) or mbk-2(RNAi), bacteria were seeded on 1 mM Isopropil-β-D-1-tiogalattopiranoside (IPTG) plates and worms were fed from L4 at 25 °C.

For par-1(RNAi), the T444T vector (Addgene plasmid # 113081; http://n2t.net/ Addgene:113081; RRID:Addgene_113081) (4) was used to achieve a stronger PAR-1 depletion. Exon 9 of par-1 was amplified using the oligos FPR97-F (ATGGCTAGCCTCAACCACACCAGCAA) and FPR97-R (CAGCCCGGGTGTTGACTTCTTTGTTGCTCT) and cloned into the Nhel and XmaI sites of the T444T vector. T444T and par-1(RNAi) bacteria were first induced in 1 mM IPTG and then seeded on 3 mM IPTG plates. mCh::mex-5 and mCh::plk-1 worms were grown on the T444T bacteria and the mCh::plk-1;par-1(zu310ts) strain was grown on par-1(RNAi) bacteria from L1 at 25°C. At L4, worms were transferred to a fresh RNAi plate.

To achieve a partial run-down of MEX-5 over time, bacteria were induced in 1 mM IPTG and seeded on 1 mM IPTG plates.

mCh::mex-5 and mCh::plk-1 worms were fed from L1 or L4 at 20°C, then imaged every 1-1.5 hours. The

The PAM sites are underlined and the synonymous mutations are in bold, the mCherry sequence can be found at https://www.sunybiotech.com/.
**mCh::mex-5** strain was used as control condition to estimate the relative reduction of MEX-5 signal with respect to the L4440 fed worms (“ctrl(RNAi)”). The intensity of the signal was scored in the anterior before cytokinesis or at 2-cell stage. To evaluate the gradient dynamics at different time-points, the PLK-1 gradient was measured from single-frame pictures and from movies of embryos of the mCh::plk-1 in ctrl(RNAi) or mex-5(RNAi) worms. The measurements were pooled according to the level of MEX-5 reduction. Since partial RNAi resulted in variable MEX-5 depletion, we analyzed the gradient of embryos in which the signal was depleted between 45 to 75% compared to the control.

**Gradient analysis**

For time lapse recordings, a Nikon ECLIPSE Ni-U microscope, equipped with a Nikon DS-U3 Digital Camera, was used. A gain of 8-10% was used for the fluorescent light. Images were taken every 10 seconds, till the completion of the first division (around 20 minutes). mCherry embryos were exposed for 800 ms. Embryos from the plk-1::sgfp strain were exposed for 400 ms. Imaging was performed in a temperature-controlled room (22°C). For gradient measurements, worms were dissected in Egg Buffer (118 mM NaCl + 48 mM KCl + 2 mM CaCl2 + 2 mM MgCl2 + 25 mM HEPES, pH 7.6) and the embryos were mounted on 3% noble agar pads immediately before imaging.

We studied gradient formation as function of time (Fig. 1A) by analyzing time-lapse movies (Movie S1A and S1B) using an ad hoc macro (available in Github, see Code availability) written with the image analysis software Fiji (6). If the position of the embryos moved during the timelapse, the Fiji plugin “StackReg” (7) was applied before the analysis to fix the embryo position. We extracted the signal intensity profile along a ROI line, which we manually drew from anterior to posterior, to avoid the cortical blebbing, the pronuclei, and, for PLK-1, the mitotic structures displaying a signal. We drew the line far from the cortex, to avoid the decrease of the signal intensity from regions with a lower thickness. We performed the analysis for each timeframe of the time-lapse recording, providing the signal distribution change as a function of time. We analyzed the resulting profiles using a custom-made Python script (available in Github): for each timeframe, the intensity was normalized to 1 using the maximum intensity value. The x-axis was also renormalized to 1 by dividing for the segment length. We applied a correction for the decrease of the signal intensity near the cortex seen in some profiles by removing the points at the beginning and end of the distribution, for which there was a deviation from the distribution mean larger than 1σ at the anterior and 2σ at the posterior.

We fitted the concentration profiles for all the timeframes using a linear function and extracted the linear coefficient as an indicator of the gradient strength, for all the timeframes. After reaching the maximum steepness (the minimum in the curve), the PLK-1 gradient began to flatten at cytokinesis. After cell division, the intensity profile displayed a two-step function, associated with rather uniform protein concentrations within the daughter cells. Starting from this point, a linear fit no longer approximated the PLK-1 intensity profile. The time to achieve the maximum gradient (and the steady state) was estimated as the frame in which the gradient displayed a minimum (if followed by a decrease in the absolute value) or reached a plateau.

**Quantification of PLK-1 and MEX-5 intensities at the 2-cell stage**

The signal in the AB and P1 cells was analyzed by taking two squared ROIs manually drawn within the whole volume of each of the two cells, avoiding pronuclei regions. Quantification of the ROI mean intensity was carried out using Fiji (8). The average of the two measurements in AB and P1 was calculated, called respectively A and P signal, and the relative increase in the AB cell was calculated according to the following formula:

\[
\frac{(A-P)}{P} \cdot 100\% 
\]  

(S1)

We pooled and averaged the values obtained from different embryos and plotted the results as mean +/- standard deviation (SD).

**Fluorescence Recovery After Photobleaching (FRAP)**

Embryos were dissected into M9 (86 mM NaCl, 42 mM Na2HPO4, 22 mM KH2PO4, and 1 mM MgSO4) on a coverslip, to which approximately one hundred 20-µm polystyrene beads were added (Bangs Laboratories) (3, 9). The coverslip was mounted on a slide and sealed with Vaseline. Embryos at pre-polarization and NEBD stages were selected. Images were acquired on a spinning disk confocal microscope controlled by the Slidebook software package (Intelligent Imaging Innovations, Denver, CO) and built on a Zeiss Axio Observer Z.1 equipped with a Zeiss Plan-Apochromat 63x/1.4NA oil immersion objective, a CSU-X1 spinning disk (Yokogawa, Tokyo, Japan) and an Evolve 512X512 EMCCD camera (Photometrics, Tucson, AZ). FRAP was performed using a Phasor photomanipulation unit (Intelligent Imaging Innovations), which delivered 488 nm light to a single circular ROI of 2.5-µm radius drawn either in the anterior or in the posterior region of the embryo. Twenty frames (1 frames corresponded to ~150 ms) before bleaching were acquired.
using a 561 nm laser (100% laser power) to evaluate the intensity baseline in the ROI. The ROI was then bleached using a 488 nm laser (100% laser-power) for 120 ms, and subsequently imaged for another 280 frames using a 561 nm laser (80% laser power). The camera intensification was 300 (out of 1000) and the gain was 2. The whole imaging time lasted around 1 minute, in a temperature-controlled room at 22°C.

The apparent diffusion coefficient \( D_c \) was calculated using the following equation (S2), which was established to study protein diffusion via FRAP in lamellae of adherent mammalian cells (10):

\[
D_c = \frac{(r_0^2 + r_{eff}^2)}{B \cdot \tau_{1/2}} \tag{S2}
\]

where \( \tau_{1/2} \) is the half-time of recovery, \( r_0 \) is the nominal radius of the bleached area (Fig. S2A, left), kept fixed to 2.5 \( \mu \text{m} \), and \( r_{eff} \) is the effective radius (Fig. S2A, right). The effective radius was obtained by extrapolating in Fiji (8) the intensity profile over a line crossing the bleached nominal ROI in the timeframe post-FRAP, and fitting it using a gaussian distribution (Fig. S2A, right). It was defined as the radius at 0.86 \( h \), where \( h \) is the height of the gaussian profile, as in (10).

The signal intensity in the nominal ROI as a function of time was automatically provided by the acquisition software (Slidebook, Intelligent Imaging Innovations), after subtraction of the background (ROI outside the embryo) and correction of the photo-bleaching during recovery by normalization to a fluorescence intensity within a ROI far from the bleach region. The curves were translated by subtracting the value of the first timeframe immediately post-FRAP (to have the first post-FRAP value set to 0), and then re-normalized to the average of the intensity values pre-FRAP. From these curves, the half-time \( \tau_{1/2} \) was extrapolated by fitting in GraphPad Prism (version 9.1.2) the resulting curves using the following 1-phase association, non-linear regression function (Fig. S2B):

\[
I(t) = \text{Plateau} \cdot (1 - e^{-\frac{t}{\tau_{1/2}}}) \tag{S3}
\]

The \( D_c \) from the individual measurements were pooled and averaged, and results were plotted as mean +/- SD.

**Calibration of particle velocity from the \( D_c \).**

The relationship between the experimental \( D_c \) and the velocity \( \vec{v} \) of the single proteins was studied using Monte Carlo simulations. Particles were generated in a point-like source (see Fig. S2C, lower panel), and then they diffused concentrically in the 3D space, according to the particle velocity. This motion is governed by the formula:

\[
\sigma^2 = D_c \cdot dt \tag{S4}
\]

with \( dt \) the simulated time-step and \( \sigma^2 \) the width of the gaussian profile of the particle distribution in one dimension (Fig. S2C, upper panel). The velocity was an input of the code. Using equation (S4), knowing \( dt \) and extracting the standard deviation of the gaussian profile of the particle spatial distribution, we obtained the \( D_c \) as the linear coefficient of the linear fit of the displacement vs time (Fig. S2D). By tuning the input velocity, we obtained a calibration curve for \( D_c \) as a function of the velocity (Fig. S2E), that was fitted by the function:

\[
D_c = -8 \cdot 10^{-4} \cdot v + 0.125 \cdot v^2 \tag{S5}
\]

Since the experimental \( D_c \) for MEX-5 was an average (\( D_{c,\text{aver}} \)) resulting from the presence of both the fast and slow components, it could be expressed as a function of the species percentages:

\[
D_{c,\text{aver}} = \%_{\text{fast}} \cdot D_{c,\text{fast}} + \%_{\text{slow}} \cdot D_{c,\text{slow}} \tag{S6}
\]

where \( D_{c,\text{fast/slow}} \) were the diffusion coefficients for MEX-5\text{}/MEX-5\text{,} respectively (to be calculated), and \( \%_{\text{fast/slow}} \) were the respective percentages at particular timepoint of cell division. The equation was solved at NEBD, for the anterior and posterior, using, at anterior, \( \%_{\text{fast}} = 30\% \) and \( \%_{\text{slow}} = 70\% \) for MEX-5\text{,} and MEX-5\text{,} respectively, and 50\% for MEX-5\text{,} and MEX-5\text{,} at posterior, as previously described (11).

We used the value of \( D_{c,\text{aver}} = 1 \ \mu\text{m}^2/\text{s} \) for the anterior and \( D_{c,\text{aver}} = 1.65 \ \mu\text{m}^2/\text{s} \) at the posterior (first value that guaranteed a positive \( D_c \)). By solving this set of equations, we got \( D_{c,\text{fast}} \sim 3 \ \mu\text{m}^2/\text{s} \) and \( D_{c,\text{slow}} \sim 0.03 \ \mu\text{m}^2/\text{s} \) for the MEX-5\text{,} and MEX-5\text{,} respectively, which correspond to the input velocities of \( v_f = 4.9 \ \mu\text{m}/\text{s} \) and \( v_s = 0.45 \ \mu\text{m}/\text{s} \),
respectively. The extrapolated values are consistent with measurements of MEX-5 diffusivity by Fluorescence Correlation Spectroscopy in (11). For this reason, the calculation was extended to the extrapolation of PLK-1 diffusivity. For the unbound pool of PLK-1, we considered $D_i = 4 \, \mu m^2/s$ as measured at pre-polarization, with the assumption that all PLK-1 was unbound at that stage, consistent with the fact that the PLK-1 gradient started later. We obtained a velocity of 5.3 $\mu m/s$.

2. Implementation of the Monte Carlo simulations for gradient formation

The Monte Carlo simulation tool was based on a C++ backbone, where most of the functionalities controlling the evolution of the system are implemented, and on a Python wrapper, providing a user-friendly configuration interface to exploit the available functionalities at run-time. Each protein is described by a C++ compiled class inheriting the general properties common to all particles and including specific behavior of that protein type.

The embryo was simulated as a 3D ellipsoid, with a volume of $50 \times 30 \times 30 \, \mu m^3$ (12), respectively along $x$, $y$ and $z$ (Fig. S3A). Proteins were simulated as point-like structures (hereby called “particles”). We created C++ classes for the two proteins of interest, PLK-1 and MEX-5. We however designed the framework to be easily extended to implement other proteins of interest. A particle (protein) manager controlled the particles by dispatching commands, such as the function “Move”, and centralizing operations common to the whole particle pool, such as random generators. With this, we minimized the memory footprint. The particle manager was also responsible for the parallelization of CPU-consuming operations, by exploiting multithreads (threads from standard library were used): the full number of available cores was exploited, and particle histories were shared among available cores.

Particle features

Several features were implemented to define the general protein behavior, and they were shared between particle classes. In the following, a list of the main properties and functions that were introduced to simulate MEX-5 and PLK-1 dynamics in the one-cell embryo.

- Initial particle concentration: this parameter was defined to take into account inhomogeneities in the particle concentration at pre-polarization. For MEX-5 we kept the parameters fixed to 70% MEX-5, and 30% MEX-5$_t$ (11), while for PLK-1 we assumed that the whole population was unbound from MEX-5 at the beginning of the process.
- Particle generation: proteins were generated in the middle of the embryo, in a number that is user-defined (in the calculations of Fig. 3 and Fig. 4 we simulated 10$^6$ particles per simulation for both proteins, to reach a balance between statistical power and computational weight of the simulations).
- Particle shuffling: to randomly distribute the particles in the 3D embryonic volume, we extracted, for each of them, a random position in the canvas. We then applied the Monte Carlo “hit or miss” method to exclude positions that were outside the ellipsoidal volume. We therefore recreated an initial condition with homogeneous concentration distributions for MEX-5 (both components) and PLK-1.
- Particle labelling: both MEX-5 and PLK-1 particle could dynamically assume different “states” and switch between them. MEX-5 presented two different components, MEX-5, and MEX-5$_t$, to which we respectively assigned the label of ID=0 and ID=1. As for PLK-1, ID=0 referred to the “unbound” state. We then labelled the other two conditions in which PLK-1 could be present, in the most general case of binding to MEX-5$_{1s}$: when bound to MEX-5, ID=1, while when bound to MEX-5$_t$, ID=2.
- Particle motion: to reproduce the protein motion in the cytoplasm, the particles had to be assigned a momentum. The apparent diffusion coefficient is a measurement of the number of proteins diffusing per unit surface and unit time and represents an average measurement of protein mobility. As such, it does not define the property of the single particles. In the Monte Carlo code, on the other hand, we simulated the motion of each single protein, regulated by its own velocity $\vec{v}$ in space. We therefore calibrated the velocity of each particle species to reproduce their experimental $D_i$ (see above and Fig. S2). The randomly generated particles (Fig. S3A) were made to diffuse in the 3D space according to Brownian motion, along directions that were chosen by extraction of random 3D polar coordinates: for each simulated time-step, two random numbers were extracted between 0-2$\pi$ and 0-$\pi$ respectively, corresponding to the polar angles $\theta$ and $\phi$. The three different components of the translational vector $\vec{r}$ were defined as:

\begin{align}
\vec{x} &= \vec{r} \cdot \sin \theta \cdot \cos \phi, \\
\vec{y} &= \vec{r} \cdot \sin \theta \cdot \sin \phi, \\
\vec{z} &= \vec{r} \cdot \cos \theta,
\end{align}

(S7) (S8) (S9)
where the radius $\vec{r}$ was defined as $\vec{r} = \vec{v} \cdot dt$, i.e. the velocity $\vec{v}$ of the particle times the simulated time-step $dt$ (1 s). Proteins were not allowed to exit the embryonic volume, to avoid particle leakage. After the selection of the direction of the motion, particles followed a linear path. If the particles were to cross the volume boundaries, we recalculated the path length $r'$ by iteratively shortening the time-step $dt$ of a factor of 1.01, until $r'$ was inside the embryo (new final particle position at the cortex). We then made the particle bouncing back inwards, for a path length equal to the missing difference $r'' = (r - r')$. To do that, a new set of polar angles $\theta'$ and $\phi'$ was iteratively extracted to satisfy the requirement: $r''$ within the embryonic volume.

- Reaction rates: both proteins changed their state according to reaction rates variable with time and position in the embryo. At every simulated timepoint, random numbers were extracted and directly compared to the reaction rates in the specific particle position at the moment of the interrogation, to see if the state had to be flipped or not. This algorithm was applied to all simulated particles. More details for the implementation of the different reaction rate are provided below, for each protein.

**Switch between states – MEX-5**

The switch between MEX-5 states was iteratively done for each MEX-5 particle and after each simulated $dt$, according to the reaction rates $k_{PAR-1}$ and $k_{PP2}$ (Fig. S3B). Despite the fact that phosphorylation by PAR-1 has been proposed to regulate the switch in MEX-5 D0, by regulating MEX-5 binding to RNAs (11-13), we made the assumption that the PAR-1/PP2 reactions directly caused the change in MEX-5 diffusivity; this allowed us to neglect the different RNA complexes and their dynamics.

The PP2A phosphatase activity was simulated as uniformly distributed throughout the cytoplasm (11) (Fig. S3C). We instead implemented the PAR-1 kinase activity as a linear function, increasing in steepness from anterior to posterior dynamically over a time range of 300 s (14) (Fig. S3C). At the beginning of the simulations, polarity establishment had not initiated and PAR-1 activity was assumed to take place uniformly in the cytoplasm with a low kinase rate ($k_{PAR-1,low}$, e.g. equal to 0.02 s$^{-1}$ if we use the value from (11)). During the first simulated 300 s (14), PAR-1 progressively accumulates in the posterior (every one second), leading to the creation of the PAR-1 gradient from the anterior value $k_{PAR-1,low}$ to posterior value $k_{PAR-1,app}$ (Fig. S3C).

**Switch between states – PLK-1**

For PLK-1 the algorithm was more complex, to take into account all the different possible scenarios that we envisaged.

1) **Binding to MEX-5**

To simulate different binding scenarios, we allowed the possibility to selectively choose PLK-1’s binding partner through the use of Boolean flags. Three scenarios were allowed, with PLK-1 binding to either MEX-5, or to MEX-5, or to both species (MEX-5). The position-dependent binding rate $k_{bind}$ was dependent on the local concentration of the binding partner(s), calculated as the number of binding partner particles in a region of 1 μm$^2$ around the interrogated PLK-1 particle position, normalized to the number of whole MEX-5 particles simulated. The factor of proportionality was called multiplication factor $M$, and was kept fixed to 1000 in our simulations. Thus:

$$k_{bind} = M \cdot C_{partner(s)} \tag{S10}$$

If an extracted random number was lower than this calculated rate, then PLK-1 bound, otherwise it would stay unbound till the next interrogation step, when the procedure was iterated. In case binding to MEX-5 was permitted, the sum of the concentrations of both components was considered. As the $k_{bind}$ to MEX-5, and MEX-5 are not known, we assumed it to be the same independently on which MEX-5 component PLK-1 was binding (Fig. S4A). To select the binding partner for the specific PLK-1 particle, we considered the relative abundance of the two MEX-5 species in that specific location. We therefore interrogated the ratio between fast and slow MEX-5 components to randomly select the partner.

2) **Free unbinding**

With free unbinding we denominated a detachment process independent of MEX-5 state. With this we aimed at recreating a very dynamic situation where the two proteins were able to bind, but also to detach from each other, in a continuous exchange with the cytoplasmic unbound PLK-1 pool (Fig. S4A). In the Result section, we show that $k_{off}$ rate had to be constant and $\sim 0.1$ s$^{-1}$. A free unbinding rate of 0 s$^{-1}$ allows to deactivate this kind of unbinding in the simulation.

3) **Imposed unbinding**

Barbieri et al.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms
With this term we referred to a form of unbinding that could be driven by conformational changes in MEX-5 structure, when switching from a state (e.g. de-phosphorylated to phosphorylated or vice versa) (Fig. S4A). We allowed to switch ON or OFF this unbinding scenario by changing Boolean flags. When activated, PLK-1 was able to return to the unbound pool every time the partner changed its state. This switch was regulated by PAR-1 kinase activity ($k_{PA R-1}$) and PP2A phosphatase activity ($k_{P P 2}$), described above.

Software replica of the readout and analysis pipeline

As in epifluorescence microscopy the light detected is coming not only from the in-focus plane, but from a bigger thickness of the sample. We extrapolated the parameters of interest (e.g. particle concentration, velocity, etc...) from a slice $\Delta z = 5 \, \mu m$ of the ellipsoidal volume, centered at the middle plane (Fig. S3D) as done when optimizing the focus during imaging. In addition to this, we implemented an analysis pipeline mimicking the one applied to image analysis: 1) we projected the particles in the slice onto a 2D plane (Fig. S3D); 2) we extracted the particle number and characteristics along a line across the cytoplasm, far from the anterior and posterior edges (Fig. S3D); 3) we refreshed the plots every 10 s to reconstruct the whole dynamics as done in a time-lapse movie; 4) from these real-time timeframes, we extracted MEX-5 concentration profiles (and other quantitative parameters) and we fitted them with a linear function, from which the gradient can be calculated (Fig. S3E, three representative timepoints).

We exploited the versatility of the Monte Carlo tool, combining Python analysis tools and C++ classes, to perform detailed real-time data analysis and visualization, with the aim to follow the dynamics of gradient formation over time. We interrogated, at fixed time intervals of 1 second (tunable by the user), the particle manager to collect pieces of information on the particle e.g. position, identity, velocity. To visualize the results, we developed dedicated Python analysis modules providing plots and figure panels, constantly refreshed during the system evolution (see Movie S2 and S3). This was achieved by exploiting already existing frameworks, such as Matplotlib [https://matplotlib.org/citing.html]. Scipy [https://scipy.org/citing.html] and ROOT [https://github.com/root-project/root/#cite]. Interface between the Python modules and the C++ underlying backbone was achieved by means of the Python BOOST library [https://www.boost.org/doc/libs/1_75_0/libs/python/doc/html/reference/index.html].

Introduction of delay in PLK-1 gradient formation

To reproduce the delay in PLK-1 gradient formation, we altered the dynamics according to which PLK-1 bound to MEX-5, by slowing down the binding rate for particular (user-defined) time-gaps. We introduced an ad-hoc coefficient to modulate the binding rate: its value linearly increased from 0 to 100% from the timepoint $T_1$ to timepoint $T_2$. Before $T_1$ the multiplication factor was fixed to 0, basically disabling PLK-1 binding to MEX-5. After $T_2$, on the other hand, it was fixed to 100%, thus not impacting anymore the PLK-1 to MEX-5 dynamics determined by the PP2A and PAR-1 activities.

Simulation of different relative protein concentrations

In the simulation results of Fig. 3 and Fig. 4, a total protein concentration of $10^6$ particles was simulated, in a ratio of 1:1. Since the protein concentrations of MEX-5 and PLK-1 in the one-cell embryo are not known, we integrated the relative concentration of the two proteins in each voxel of our geometrical volume, to finally determine the likelihood of binding. The algorithm then determined:

- if PLK-1 bound or not to MEX-5, stochastically, according to the binding rate $k_{b}b_{i}d$;
- if the binding was supposed to happen, then the local concentrations of MEX-5 and PLK-1 were calculated: in each voxel of the embryonic volume, the binding between the two is allowed only if there is an unbound MEX-5 protein. If the number of PLK-1 particles bound to MEX-5 was equal to the number of MEX-5 particles in the voxel, no further binding was allowed.

- The algorithm iteratively probed all PLK-1 particles in each voxel of the embryo.

The individual number of MEX-5 and PLK-1 particles were now set separately, which allowed us to change their relative concentration.

Results from this version of the algorithm were benchmarked by comparing to the previously described simulations (conditions: $10^6$ MEX-5 and PLK-1 particles, binding to MEX-5, imposed unbinding ON and $k_{off}=0.1s^{-1}$, $T_1=350\, s$ and $T_2=800\, s$), and no difference was observed between the two simulation algorithms.

3. Summary of parameters and scenario choices

We recapitulate the parameters used in the Monte Carlo code in Table S2. Illustrative schematics of the different possible biological scenarios achievable with our computational approach are shown in Fig. S3F for MEX-5 (left) and for PLK-1 (right).
4. Statistical analysis

For experimental analysis, each condition was averaged among the indicated number of embryos (N), randomly selected from the dissected worms. For the simulation results, average values and SD were derived from the individual results of at least 10 independent runs, which were run in parallel for each set of simulation settings. The statistical significance was performed using GraphPad Prism version 9.1.2 for Windows (GraphPad Software, San Diego, California USA).

Details on the test statistic (e.g., t, U and F values) and precise P values are noted in Table S2.

5. References

1. L. Martino et al., Channel Nucleoporins Recruit PLK-1 to Nuclear Pore Complexes to Direct Nuclear Envelope Breakdown in C. elegans. Dev Cell 43, 157-171.e157 (2017).
2. D. G. Morton, W. A. Hoose, K. J. Kemphues, A genome-wide RNAi screen for enhancers of par mutants reveals new contributors to early embryonic polarity in Caenorhabditis elegans. Genetics 192, 929-942 (2012).
3. Y. Wu, H. Zhang, E. E. Griffin, Coupling between cytoplasmic concentration gradients through local control of protein mobility in the Caenorhabditis elegans zygote. Mol Biol Cell 26, 2963-2970 (2015).
4. J. Pellettieri, V. Reinke, S. K. Kim, G. Seydoux, Coordinate activation of maternal protein degradation during the egg-to-embryo transition in C. elegans. Dev Cell 5, 451-462 (2003).
5. C. M. Schubert, R. Lin, C. J. de Vries, R. H. Plasterk, J. R. Priess, MEX-5 and MEX-6 function to establish soma/germline asymmetry in early C. elegans embryos, Mol Cell 5, 671-682 (2000).
6. J. Schindelin et al., Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676-682 (2012).
7. P. Thévenaz, U. E. Ruttimann, M. Unser, A pyramid approach to subpixel registration based on intensity. IEEE Trans Image Process 7, 27-41 (1998).
8. C. T. Rueden et al., ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18, 529 (2017).
9. F. B. Robin, W. M. McFadden, B. Yao, E. M. Munro, Single-molecule analysis of cell surface dynamics in Caenorhabditis elegans embryos. Nat Methods 11, 677-682 (2014).
10. M. Kang, C. A. Day, A. K. Kenworthy, E. DiBenedetto, Simplified equation to extract diffusion coefficients from confocal FRAP data. Traffic 13, 1589-1600 (2012).
11. E. E. Griffin, D. J. Odde, G. Seydoux, Regulation of the MEX-5 gradient by a spatially segregated kinase/phosphatase cycle. Cell 146, 955-968 (2011).
12. B. R. Daniels, T. M. Dobrowsky, E. M. Perkins, S. X. Sun, D. Wirtz, MEX-5 enrichment in the C. elegans early embryo mediated by differential diffusion. Development 137, 2579-2585 (2010).
13. D. Tavella, A. Ertekin, H. Schaal, S. P. Ryder, F. Massi, A Disorder-to-Order Transition Mediates RNA Binding of the Caenorhabditis elegans Protein MEX-5. Biophys J 118, 2001-2014 (2020).
14. R. Ramanujam, Z. Han, Z. Zhang, P. Kanchanawong, F. Motegi, Establishment of the PAR-1 cortical gradient by the aPKC-PRB circuit. Nat Chem Biol 14, 917-927 (2018).
Supplementary Figures

A) Average absolute values of the gradient for the \textit{plk-1::sgfp} strain, extrapolated at different stages of the first asymmetric cell division. The percentages reported close to each bar represent the relative gradient with respect to the maximum gradient.

B) Time after which the maximum gradient is achieved for \textit{plk-1::sgfp} early-stage embryos.

C) Average experimental curve for PLK-1 gradient as a function of time for \textit{plk-1::sgfp} early embryos.

D) Relative increase of the signal in the AB cell with respect to the P1 cell for the \textit{plk-1::sgfp}, calculated using \textbf{equation (S1)}. The number N of analyzed embryos is reported next to the curve.

E) Apparent diffusion coefficients for the \textit{mCh::plk-1} (from Fig. 2A) and \textit{the plk-1::sgfp} strains at NEBD. Statistical significance was obtained with 2-way Anova test, with Tukey’s multiple comparison.

In the bar graphs of all Supplementary Figures, bars represent mean values, dots individual measurements while error bars represent the standard deviation (SD). The number of analyzed embryos is specified for each condition in all panels. For all Supplementary Figures, ns: non-statistically significant; *: p<5x10^{-2}; **: p<10^{-2}; ***: p<10^{-3}; ****: p<10^{-4}. The statistical analysis for all figures is summarized in Table S2.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms.

Fig. S2. Relationship between protein velocity and apparent diffusion coefficient $D_c$.

A) Left: schematic representation of a one-cell embryo with a bleached area (black circle) and its nominal radius. Right: intensity line profile over of the nominal radius and representation of how the effective radius was calculated (from (10)).

B) Example of MEX-5 experimental FRAP recovery curve and representation of how the half-time is extrapolated.

C) Simulation of particle diffusion starting from a single point in 3D space (lower panel). The velocity is an input of the code. The gaussian profile of the particle distribution in 1-dimension is extrapolated (upper panel).

D) Plot of the squared displacement from the gaussian profile ($\sigma^2$) as a function of the time. The $D_c$ is extrapolated as the linear coefficient of the linear fit of this graph.

E) Relationship between the apparent diffusion coefficient and velocity. The function used to fit the datapoints is reported in the image.
Barbieri et al.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms
Fig. S3 Schematics of Monte Carlo implementation.

A) The *C. elegans* embryo was simulated as an ellipsoid of size: 50 x 30 x 30 µm³. Proteins were randomly generated and homogenized in the volume. They diffused according to random Brownian motion within the volume, and they stochastically changed their state according to bio-chemical reaction rates.

B) MEX-5 particles stochastically switched their state (MEX-5ᵣ → MEX-5ₛ or vice versa), depending on the reaction rates in the particle position. $k_{PAR-1}$: PAR-1 kinase rate, $k_{PP2}$: PP2A phosphatase rate.

C) PAR-1 rate $k_{PAR-1}$ formed a posterior gradient over 300 seconds, while the PP2 rate $k_{PP2}$ was uniformly distributed along the embryo axis throughout the cell division.

D) Reproduction of the chosen detection technique and final readout: a volume slice Δz, centered at the middle plane of the ellipsoid (delimited by blue planes) was considered, to mimic the microscope depth of focus. The particles in the slice were projected onto a 2D plane. Quantitative parameters were extracted along a ROI line (black) crossing the embryo from anterior to posterior, as done during the time-lapse processing and analysis.

E) Representative images of simulated MEX-5 concentration profile along the drawn ROI line at specific time-points. The orange line represents the applied linear fit, to extrapolate the linear coefficient as indicator.

F) Schematics of the different scenarios that can be implemented and simulated for the biochemical reactions affecting MEX-5 (left), and the binding/unbinding involving PLK-1 and MEX-5 (right). In the violet boxes the reaction rates, in the green boxes the time and in the black ones the proteins.
Fig. S4. PLK-1 simulation scenarios and concentration of different PLK-1 pools over time along the embryo axis.
A) Scheme of simulated PLK-1 binding and unbinding scenarios. Binding to MEX-5, only, to MEX-5; only (green), or to MEX-5fs (red) were implemented. The free unbinding (blue) and the imposed unbinding (violet) were implemented.
B) 2D distribution of PLK-1 binding rate as a function of time and position along the embryo axis. Different time-frames are shown according to the color legend, from pre-polarization (red) to maximum gradient (blue). Simulations were performed by using settings in which binding to MEX-5fs and both unbinding modalities (free and imposed) were allowed and results are the average of 10 simulation runs.
C) Simulation results on the 3D distribution of the different PLK-1 pools: “Unbound PLK-1” (top), “PLK-1 bound to MEX-5,” (middle) and “PLK-1 bound to MEX-5fs” (bottom), as a function of time and position along the embryo axis. Results are average values from 10 simulation runs. Simulations were performed allowing binding to MEX-5fs and by using both unbinding modalities.
D) Relative difference between experimental (orange) and simulated (green) gradients, calculated using equation (2), as a function of time.
Fig. S5. *mex-5(RNAi)* reduced PLK-1 asymmetry.

A) Decrease of MEX-5 signal intensity in the *mCh::mex-5* strain after *mex-5(RNAi)*. Data with a MEX-5 reduction between 45-75% were pooled together. Statistical significance was obtained with 2-tail unpaired t-test.

B) Average values of the gradient in the *mCh::plk-1* strain, after *mex-5(RNAi)*, extrapolated at different stages of the first asymmetric cell division (shown on the y axis). Statistical significance between control and treated conditions, for each separate stage, was obtained with 2-tail unpaired t-test.

C) Relative difference of PLK-1 intensity between AB and P1 in the *mCh::plk-1* strain, normalized to AB, after *mex-5(RNAi)*. Statistical significance was obtained with unpaired two-tailed Mann-Whitney test.
Supplementary Tables

Table S1. MEX-5 and PLK-1 diffusion coefficients.

|                        | Non-treated strains | RNAi conditions |
|------------------------|---------------------|-----------------|
|                        | mCh::mex-5 (OP50 fed) | mCh::plk-1 (OP50 fed) |
| Pre-polarization       | Ant. vs Post. Ratio (P:A) | Ant. vs Post. Ratio (P:A) |
|                        | 0.84±0.29 vs 0.82±0.26 0.98 | 3.36±1.28 vs 3.50±1.35 1.04 |
| NEBD                   | 1.00±0.28 vs 1.90±0.94 1.9 | 1.88±0.53 vs 3.27±0.73 1.74 |
| RNAi conditions        | Ant. vs Post. Ratio (P:A) | Ant. vs Post. Ratio (P:A) |
| NEBD                   | 0.85±0.33 vs 1.50±0.50 1.76 | 1.16±0.34 vs 1.69±0.58 1.46 |
|                        | 2.00±0.70 vs 2.12±0.73 1.06 |  |
| NEBD                   | 2.61±1.21 vs 2.33±0.99 0.89 |  |
| NEBD                   | 1.00±0.28 vs 1.52±0.44 1.52 | 1.37±0.36 vs 1.87±0.53 1.37 |
| Simulated average velocity | MEX-5 | PLK-1 |
| Pre-polarization       | Ant. vs Post. Ratio (P:A) | Ant. vs Post. Ratio (P:A) |
|                        | ~1.5 vs ~1.6 1.07 | ~4.2 vs ~4.2 1 |
| NEBD                   | ~1.5 vs ~2.8 1.87 | ~3.6 vs ~4.8 1.33 |
| MEX-5 in par-1(RNAi)   | PLK-1 in par-1(RNAi) |
| Pre-polarization       | Ant. vs Post. Ratio (P:A) | Ant. vs Post. Ratio (P:A) |
|                        | ~1.8 vs ~1.7 0.94 | ~4.4 vs ~4.5 1.02 |
| NEBD                   | ~1.7 vs ~1.8 1.05 | ~4.2 vs ~4.3 1.02 |

Diffusion coefficients of MEX-5 and PLK-1 as extrapolated from the FRAP experiments for the different conditions reported in Fig. 2. The average velocity as extrapolated from the simulations for untreated and par-1(RNAi) embryos is also reported.
Table S2. Statistical analysis of all main and supplementary Figures.

| Fig. 1A | Stage                | P  | DF | t   |
|---------|----------------------|----|----|-----|
| Early embryos        | 0.515 | 30  | 0.66|
| Relax. at P          | 0.055 | 57  | 1.96|
| Ruffling             | < 10E-6 | 70  | 6.14|
| PC                   | < 10E-6 | 83  | 9.94|
| PNM                  | < 10E-6 | 85  | 14.27|
| PN centr.            | < 10E-6 | 85  | 12.89|
| Steady state         | < 10E-6 | 85  | 17.01|

| Fig. 1B | P | DF | t |
|---------|---|----|---|
| 6 x 10E-4 | 30 | 3.84 |

| Fig. 1D | P | Sum of ranks in mCh::mex-5, mCh::plk-1 | U |
|---------|---|----------------------------------------|---|
| < 10E-6 | 634, 1319 | 4 |

| Fig. S1E | F (DFn, DFd) | P |
|----------|--------------|---|
| Interaction | F (1, 64) = 0.4350 | 0.5119 |
| Row (position) | F (1, 64) = 71.73 | < 10E-4 |
| Column (stage) | F (1, 64) = 8.582 | 0.0047 |

| Tukey's test | Predicted (LS) mean diff. | 95% CI of diff. | Adjusted P |
|--------------|---------------------------|------------------|------------|
| Ant:plk-1::sgfp vs Ant:mCh::plk-1 | -0.639 | -1.289 to 0.01086 | 0.0556 |
| Ant:plk-1::sgfp vs Post:plk-1::sgfp | -1.625 | -2.396 to -0.8550 | <0.0001 |
| Ant:plk-1::sgfp vs Post:mCh::plk-1 | -2.029 | -2.708 to -1.351 | <0.0001 |
| Ant:mCh::plk-1 vs Post:plk-1::sgfp | -0.9864 | -1.636 to -0.3365 | 0.0009 |
| Ant:mCh::plk-1 vs Post:mCh::plk-1 | -1.391 | -1.928 to -0.8531 | <0.0001 |
| Post:plk-1::sgfp vs Post:mCh::plk-1 | -0.4041 | -1.082 to 0.2741 | 0.4018 |

| Fig. 2A | F (DFn, DFd) | P |
|---------|--------------|---|
| Interaction | F (1, 77) = 15.15 | 0.0002 |
| Row (position) | F (1, 77) = 14.02 | 0.0003 |
| Column (stage) | F (1, 77) = 27.57 | P<0.0001 |

| Tukey's test | Predicted (LS) mean diff. | 95% CI of diff. | Adjusted P |
|--------------|---------------------------|------------------|------------|
| Ant:Pre vs Ant:NEBD | -0.1609 | -0.6033 to 0.2815 | 0.7752 |
| Ant:Pre vs Post:Pre | 0.01752 | -0.4249 to 0.4600 | 0.9996 |
| Ant:Pre vs Post:NEBD | -1.065 | -1.502 to -0.6281 | <0.0001 |
| Ant:NEBD vs Post:Pre | 0.1784 | -0.2640 to 0.6209 | 0.7153 |
| Ant:NEBD vs Post:NEBD | -0.9044 | -1.342 to -0.4672 | <0.0001 |
| Post:Pre vs Post:NEBD | -1.083 | -1.520 to -0.6456 | <0.0001 |
Barbieri et al.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms

### Fig. 2B

| Interaction               | F (DFn, DFd) | P     |
|---------------------------|--------------|-------|
| Row (position)            | F (1, 87) = 12.60 | 0.0006 |
| Column (stage)            | F (1, 87) = 15.56 | 0.0002 |

**Tukey's test**

| Interaction                  | Predicted (LS) mean diff. | 95% CI of diff. | Adjusted P |
|-----------------------------|---------------------------|-----------------|------------|
| Ant:Pre vs Ant:NEBD         | 1.476                     | 0.6967 to 2.255 | < 10E-4    |
| Ant:Pre vs Post:Pre         | -0.1447                   | -0.9468 to 0.6573 | 0.9649     |
| Ant:Pre vs Post:NEBD        | 0.08534                   | -0.7456 to 0.9163 | 0.9931     |
| Ant:NEBD vs Post:Pre        | -1.621                    | -2.390 to -0.8507 | < 10E-4    |
| Ant:NEBD vs Post:NEBD       | -1.391                    | -2.190 to -0.5906 | < 10E-4    |
| Post:Pre vs Post:NEBD       | 0.23                      | -0.5922 to 1.052 | 0.8837     |

### Fig. 2C (L4440)

| Interaction               | F (DFn, DFd) | P     |
|---------------------------|--------------|-------|
| Row (position)            | F (1, 134) = 0.7644 | 0.3835 |
| Column (treatment)        | F (2, 134) = 18.12 | < 10E-4 |

**Tukey's test**

| Interaction                  | Predicted (LS) mean diff. | 95% CI of diff. | Adjusted P |
|-----------------------------|---------------------------|-----------------|------------|
| Ant:L4440 vs Ant:mex-5/6(RNAi) | -0.8378                  | -1.542 to -0.1336 | 0.0099     |
| Ant:L4440 vs Ant:mbk-2(RNAi)  | -1.447                   | -2.139 to -0.7550 | <0.0001    |
| Ant:L4440 vs Post:L4440     | -0.5319                  | -1.286 to 0.2225 | 0.3263     |
| Ant:L4440 vs Post:mex-5/6(RNAi) | -0.9621                 | -1.706 to -0.2179 | 0.0037     |
| Ant:L4440 vs Post:mbk-2(RNAi) | -1.164                   | -1.855 to -0.4718 | <0.0001    |
| Ant:mex-5/6(RNAi) vs Ant:mbk-2(RNAi) | -0.6091               | -1.278 to 0.05948 | 0.0962     |
| Ant:mex-5/6(RNAi) vs Post:L4440 | 0.3059                  | -0.4273 to 1.039 | 0.8331     |
| Ant:mex-5/6(RNAi) vs Post:mex-5/6(RNAi) | -0.1243              | -0.8470 to 0.5983 | 0.9962     |
| Ant:mex-5/6(RNAi) vs Post:mbk-2(RNAi) | -0.3258               | -0.9944 to 0.3427 | 0.7215     |
| Ant:mbk-2(RNAi) vs Post:L4440 | 0.915                   | 0.1937 to 1.636 | 0.0047     |
| Ant:mbk-2(RNAi) vs Post:mex-5/6(RNAi) | 0.4848               | -0.2259 to 1.195 | 0.3636     |
| Ant:mbk-2(RNAi) vs Post:mbk-2(RNAi) | 0.2833                | -0.3723 to 0.9389 | 0.8116     |
| Post:L4440 vs Post:mex-5/6(RNAi) | -0.4302               | -1.202 to 0.3415 | 0.5922     |
| Post:L4440 vs Post:mbk-2(RNAi)  | -0.6317                 | -1.353 to 0.08960 | 0.1222     |
| Post:mex-5/6(RNAi) vs Post:mbk-2(RNAi) | -0.2015              | -0.9122 to 0.5091 | 0.9634     |

### Fig. 2C (T444T)

| Interaction               | F (DFn, DFd) | P     |
|---------------------------|--------------|-------|
| Row (position)            | F (1, 72) = 11.03 | 0.0014 |
| Column (treatment)        | F (1, 72) = 2.365 | 0.1285 |

---

Barbieri et al.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms
### Tukey's test

|                          | Predicted (LS) mean diff. | 95% CI of diff.         | Adjusted P |
|--------------------------|---------------------------|-------------------------|------------|
| Ant:par-1(RNAi) vs Ant:T444T | 0.4012                    | 0.01758 to 0.7847       | 0.0369     |
| Ant:par-1(RNAi) vs Post:par-1(RNAi) | 0.1833                  | -0.1889 to 0.5556       | 0.5689     |
| Ant:par-1(RNAi) vs Post:T444T | -0.09847                   | -0.4707 to 0.2738       | 0.8984     |
| Ant:T444T vs Post:par-1(RNAi) | -0.2178                    | -0.6103 to 0.1747       | 0.4671     |
| Ant:T444T vs Post:T444T | -0.4996                     | -0.8921 to -0.1071      | 0.007      |
| Post:par-1(RNAi) vs Post:T444T | -0.2818                    | -0.6633 to 0.09963      | 0.2195     |

### Fig. 5C

| P | Sum of ranks in ctrl(RNAi) and mex-5(RNAi) | U |
|---|---------------------------------------------|---|
| < 10E-6 | 52299 , 21621 | 5331 |

### Fig. S5A

| P | DF | t |
|---|----|---|
| < 10E-6 | 378 | 30.73 |

### Fig. S5B

#### Stage

| P | DF | t |
|---|----|---|
| Early embryos | 0.17213 | 45 | 1.39 |
| Relax. at P | 0.33351 | 50 | 0.98 |
| Ruffling | 0.441743 | 77600.77 | 6.14 |
| PC | 0.004721 | 90 | 2.9 |
| PNM | 0.016536 | 85 | 2.45 |
| PN centr. | < 10E-6 | 118 | 5.87 |
| Steady state | 0.000011 | 93 | 4.66 |

### Fig. S5C

| P | Sum of ranks in ctrl(RNAi) and mex-5(RNAi) | U |
|---|---------------------------------------------|---|
| < 10E-6 | 20411 , 7084 | 2034 |

Results of the statistical tests performed in the different Figures of the paper, reporting precise P-values and details of the test (t, U values, degree of freedom (DF)).
Table S3. Parameters used in the Monte Carlo simulations.

| Settable parameters | Boolean flags |
|---------------------|---------------|
| Symbol              | Explanation   | Option       | Selection |
| Δz                  | Slice thickness | Binding to MEX-5s | 1=ON, 0=OFF |
| N                   | Number of MEX-5 and PLK-1 particles | Binding to MEX-5s | 1=ON, 0=OFF |
| vMEX-5s             | Velocity MEX-5s | Imposed unbinding | 1=ON, 0=OFF |
| vMEX-5f             | Velocity MEX-5f | Derived parameters |

| Vsub PLK-1          | Velocity PLK-1 unbound from MEX-5 | Formula | Explanation |
|---------------------|-----------------------------------|---------|-------------|
| C(MEX-5s)           | Initial concentration of MEX-5s t>300 s | mp PAR-1,fin = (k PAR-1,upp - k PAR-1,low)/50 | Slope of PAR-1 gradient after its establishment |
| C(MEX-5f)           | Initial concentration of MEX-5f t<300 s | mp PAR-1,1, t = t * (mp PAR-1,fin -0)/ 300 | Slope of PAR-1 gradient at a precise time t<300 s |
| kPAR-1,low          | Lower limit of PAR-1 kinase rate k PAR-1,app, t = k PAR-1,app + m *x | | Value of the upper limit of PAR-1 kinase rate at a time t<300 s |
| kPAR-1,upp          | Upper limit of PAR-1 kinase rate m prim MEX-5 = (1-0)/(T2-T1) | | Slope of the linear increase in MEX-5 availability for binding with PLK-1 |
| kPP2                | PP2 phosphatase rate | Fixed parameters |
| kbind               | PLK-1 binding rate | Values | Explanation |
| M                   | Factor of proportionality between kbind and MEX-5 concentration x=50 µm | | Embryo length along x |
| koff                | PLK-1 unbinding rate y=30 µm | | Embryo length along y |
| T1                  | Starting time for MEX-5 priming z=30 µm | | Embryo length along z |
| T2                  | Final time when all MEX-5 is primed t=300 s | | Time for PAR-1 gradient establishment |
| dt                  | Time-step for simulated temporal evolution dt=1 s | | |

Summary of the settable and fixed parameters, of the derived quantities and of the Boolean flags used for the choice of the simulation scenario. The symbols and explanations are specified for each entry, and specific values for the fixed parameters are reported.
Table S4. Parameters extrapolated from the fit of MEX-5 curves in Fig. 3C.

| Best-fit values | Simulation | Exp. average |
|-----------------|------------|--------------|
| A               | 0.4275     | 0.438        |
| B               | 0.074      | 0.0729       |
| C               | -2.875     | -3.068       |

95% CI

| Best-fit values | Simulation | Exp. average |
|-----------------|------------|--------------|
| A               | 0.423 to 0.432 | 0.432 to 0.445 |
| B               | 0.070 to 0.079 | 0.069 to 0.077 |
| C               | -3.045 to -2.718 | -3.220 to -2.926 |

Goodness of Fit

| R squared | Simulation | Exp. average |
|-----------|------------|--------------|
| 0.985     | 0.989      |              |

The table reports the values of the parameters A, B and C of the analytical function (equation (1)) used to fit the experimental and simulation data on MEX-5 gradient. The interval below represents the uncertainty at 95% confidence level. The goodness of the fit is reported in the table.

Table S5. Parameters extrapolated from the fit of PLK-1 curves in Fig. 4B.

| Best-fit values | \( k_{\text{off}} = 0.00 \text{ s}^{-1} \) | \( k_{\text{off}} = 0.01 \text{ s}^{-1} \) | \( k_{\text{off}} = 0.03 \text{ s}^{-1} \) | \( k_{\text{off}} = 0.05 \text{ s}^{-1} \) | \( k_{\text{off}} = 0.10 \text{ s}^{-1} \) | \( k_{\text{off}} = 0.50 \text{ s}^{-1} \) | Exp. average |
|-----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------|
| A               | 0.611                         | 0.411                         | 0.29                          | 0.236                         | 0.162                         | 0.044                         | 0.172        |
| B               | 0.0612                        | 0.068                         | 0.071                         | 0.07                          | 0.065                         | 0.06                          | 0.086        |
| C               | -2.925                        | -2.89                         | -3.004                        | -2.848                        | -2.84                         | -2.694                        | -5.708       |

95% CI

| Best-fit values | Simulation | Exp. average |
|-----------------|------------|--------------|
| A               | 0.604 to 0.618 | 0.407 to 0.416 | 0.286 to 0.294 | 0.233 to 0.239 | 0.160 to 0.165 | 0.040 to 0.048 | 0.170 to 0.174 |
| B               | 0.058 to 0.065 | 0.065 to 0.072 | 0.067 to 0.077 | 0.065 to 0.075 | 0.060 to 0.071 | 0.043 to 0.085 | 0.083 to 0.090 |
| C               | -3.063 to -2.795 | -3.042 to -2.747 | -3.205 to -2.819 | -3.032 to -2.678 | -3.054 to -2.645 | -3.685 to -2.005 | -5.945 to -5.485 |

Goodness of Fit

| R squared | Simulation | Exp. average |
|-----------|------------|--------------|
| 0.989     | 0.987      | 0.981        | 0.982     | 0.973     | 0.65       | 0.995      |

The table reports the values of the parameters A, B and C of the analytical function (equation (1)) used to fit the experimental and simulation data on PLK-1 gradient, in different conditions of unbinding between PLK-1 and MEX-5 (\( k_{\text{off}} \)). The interval below represents the uncertainty at 95% confidence level. The goodness of the fit is reported in the table.
Barbieri et al.
Modelling protein dynamics in C. elegans embryos reveals that the PLK-1 gradient relies on weakly coupled reaction-diffusion mechanisms

Table S6. Parameters extrapolated from the fit of PLK-1 curves in Fig. 4E and 4F.

| Best-fit values | T1=0 | T1=08 | T1=35 | T1=35 | T1=35 | T1=35 | Exp. average |
|-----------------|------|-------|-------|-------|-------|-------|--------------|
| T2=50 T2=100    |      |       |       |       |       |       |              |
| A               | 0.15 | 0.168 | 0.141 | 0.15  | 0.149 | 0.153 | 0.155       |
| B               | 0.083| 0.056 | 0.051 | 0.133 | 0.121 | 0.094 | 0.081       |
| C               | -3.816| -3.697| -3.596| -6.998| -7.053| -6.161| -5.829       |
| 95% CI          |      |       |       |       |       |       |              |
| A               | 0.147 to 0.155 | 0.160 to 0.176 | 0.132 to 0.153 | 0.147 to 0.154 | 0.146 to 0.152 | 0.149 to 0.158 | 0.149 to 0.162 | 0.170 to 0.174 |
| B               | 0.074 to 0.094 | 0.050 to 0.062 | 0.045 to 0.058 | 0.116 to 0.153 | 0.109 to 0.136 | 0.084 to 0.105 | 0.073 to 0.091 | 0.083 to 0.090 |
| C               | -4.257 to -3.436 | -4.026 to -3.411 | -3.963 to -3.287 | -8.034 to -6.150 | -7.856 to -6.363 | -6.850 to -5.571 | -6.459 to -5.285 | -5.945 to -5.485 |

Goodness of Fit

| R squared | 0.961 | 0.964 | 0.948 | 0.973 | 0.978 | 0.973 | 0.967 | 0.995 |

The table reports the values of the parameters A, B and C of the analytical function (equation (1)) used to fit the experimental and simulation data on PLK-1 gradient, in different conditions of delayed gradient formation. The interval below represents the uncertainty at 95% confidence level. The goodness of the fit is reported in the table.

Table S7. Flex points for the curves in Fig. 4F.

| Curve | 10% max. gradient | 90% max. gradient |
|-------|-------------------|-------------------|
| T1=35, T2=50 | 36.1              | 69.14             |
| T1=35 T2=65 | 40.17             | 76.99             |
| T1=35, T2=80 | 41.92             | 86.97             |
| T1=35, T2=95 | 44.39             | 95.54             |
| Exp. average | 40.67             | 90.63             |

Extrapolation of the flex points, defined as the timeframes at which the curves reach 10% and 90% of the maximum gradient.
Supplementary Movies

**Movie S1** (separate file). **Gradient formation in the mCh::plk-1 and mCh::mex-5 strains.**
Time-lapse recording of dynamic re-localization of PLK-1 (A) and MEX-5 (B) during the first asymmetric cell division in the mCh::plk-1 and mCh::mex-5 strains.

**Movie S2** (separate file). **MEX-5 gradient evolution over time.**
A) Upper panel: distribution of total MEX-5 particle concentration from pre-polarization to steady state, evolving over time during the Monte Carlo simulation. Lower left: profile of total MEX-5 concentration along a line drawn in the cytoplasm, in the volume slice \( \Delta z \). The orange line represents the linear fit of the distribution, from which the gradient is extracted. Lower right: extrapolated MEX-5 gradient plotted as a function of time.
B) Simulated distribution of de-phosphorylated MEX-5\(_i\) (upper panel) and phosphorylated MEX-5\(_f\) (lower panel), evolving over time from pre-polarization to steady state during the Monte Carlo simulation.

**Movie S3** (separate file). **PLK-1 gradient evolution over time.**
A) Upper panel: distribution of total PLK-1 particle concentration from pre-polarization to steady state, evolving over time during the Monte Carlo simulation. Lower left: profile of total PLK-1 concentration along a line drawn in the cytoplasm, in the volume slice \( \Delta z \). The orange line represents the linear fit of the distribution, from which the gradient is extracted. Lower right: extrapolated PLK-1 gradient plotted as a function of time.
B) Simulated distribution of the percentage of PLK-1 bound to MEX-5\(_i\) (upper panel), bound to MEX-5\(_f\) (middle panel) and the unbound component (lower panel), evolving over time from pre-polarization to steady state during the Monte Carlo simulation. In this simulation, binding to MEX-5\(_f+s\) was allowed and both unbinding modalities were activated (\( k_{off} = 0.1 s^{-1} \)).