

Supplemental Data

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A system of counteracting feedback loops regulates Cdc42p activity during spontaneous cell polarization

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The effect of Cdc24 on the motility of the polar cap. The nucleotide bound state of Cdc42p is regulated by the GEF Cdc24p in addition to GAPs. To assess the effect of GEF activity on polarization, Cdc24p tagged with a myristoylation signal (myr-Cdc24p) was over-expressed in *rsr1Δ* cells. As a control, a mutant form (myrG2A-Cdc24p) of the tag was used. Myristoylation of the Cdc24p is expected to result in uniform localization around the cell cortex. The polar cap spanned larger area (data not shown) in cells over-expressing myr-Cdc24p confirming the expectations of Cdc24 being uniformly targeted to the cell membrane. Over-expression of the myr-Cdc24p in *rsr1Δ* cells suppressed the mobility of the polar cap significantly, whereas the control protein (myrG2A-Cdc24p) in the same genetic background had a minor affect on the diffusion of the polar cap (Figure 3A). These data indicate that increased and membrane-localized GEF activity reduces the mobility of the waves.

Modeling spontaneous polarization. Below we propose a phenomenological model that is inspired by the experimental results presented in the main text. The goal of this model is not to describe the molecular interactions in detail, since the current knowledge on the molecular interactions is not detailed enough to build molecular models. Conversely, we present a system level model of cellular polarization during budding based on two positive feedback loops and one negative feedback loop:

Actin-independent positive feedback loop: A self-amplifying positive feedback loop (Butty et al., 2002; Shimada et al., 2004) through the interactions of Cdc24p, Bem1p, Cdc42p and possibly other membrane-bound proteins (Toenjes et al., 2004) has the capacity for spontaneous symmetry breaking (Irazoqui et al., 2003) by polymerizing all of these proteins into a polar cap.

Actin-mediated positive feedback loop: This feedback could be mediated by the actin-based delivery of secretory vesicles containing, for example, Cdc42p or Cdc24p (Wedlich-Soldner et al., 2003), which would lead to polarization in the absence of the actin-independent positive feedback loop (Irazoqui et al., 2003).

Actin-mediated negative feedback loop: Localized activated Cdc42p reorganizes the actin cytoskeleton to deliver further GAPs (inhibitors) towards the cap creating a negative feedback loop.

The following phenomenological equations describe the dynamics of the feedback regulation of the activator and the lateral diffusion of the activator in the polar cap along the cell periphery:

$$\begin{aligned} \frac{\partial A(x,t)}{\partial t} &= k_a \frac{A^2(x,t)}{A_0^2 + A^2(x,t)} B(t) - \gamma_a A(x,t) + k_b A(x,t - \Delta t) + D \frac{\partial^2 A(x,t)}{\partial x^2} \\ \frac{\partial B(t)}{\partial t} &= \left\langle \left[k_a \frac{A^2(x,t)}{A_0^2 + A^2(x,t)} B(t) - \gamma_a A(x,t) + k_b A(x,t - \Delta t) \right] \right\rangle_x \end{aligned} \quad [\text{S1}]$$

where x denotes the spatial coordinate along the cell periphery and t denotes time. A denotes the concentration of activated Cdc42p in the membrane, while B is the concentration of a cytoplasmic protein that facilitates the activation of Cdc42p (for example Bem1p). Within the cytoplasm B diffuses much faster compared to the diffusion of A in the membrane. Therefore the cytoplasm is assumed to be well stirred reflected by $\langle \dots \rangle_x$ indicating an average over position. D is the diffusion constant of Cdc42p within the polar cap. The first term indicates the actin-independent positive feedback through an autocatalytic (quadratic) activation of Cdc42p characterized by a Michaelis constant A_0 and turn-over rate k_a . The second term denotes Cdc42p inactivation with rate constant γ_a . The third term reflects the actin-mediated loop which is delayed with respect to the actin-independent loop. It is assumed that nucleation and polymerization of the actin cables followed by vesicle delivery introduces a time delay Δt with respect to the initial symmetry breaking. The rate constant k_b quantifies the strength of the actin-mediated feedback loop. If $k_b > 0$ this loop is positive whereas for $k_b < 0$ a negative loop exists.

By defining the following parameters:

$$\begin{aligned}
a &\equiv \frac{A}{A_0} \\
\tilde{t} &\equiv t\gamma_a \\
\tilde{x} &\equiv x\sqrt{\frac{\gamma_a}{D}} \\
\alpha &\equiv \frac{k_a}{A_0\gamma_a} \\
\beta &\equiv \frac{k_b}{\gamma_a}
\end{aligned}
\tag{S2}$$

equation [S1] can be reduced to:

$$\begin{aligned}
\frac{\partial a(\tilde{x}, \tilde{t})}{\partial \tilde{t}} &= \alpha \frac{a^2(\tilde{x}, \tilde{t})}{1+a^2(\tilde{x}, \tilde{t})} B(\tilde{t}) - a(\tilde{x}, \tilde{t}) + \beta a(\tilde{x}, \tilde{t} - \Delta t) + \frac{\partial^2 a(\tilde{x}, \tilde{t})}{\partial \tilde{x}^2} \\
\frac{\partial B(\tilde{t})}{\partial \tilde{t}} &= \left\langle - \left[\alpha \frac{a^2(\tilde{x}, \tilde{t})}{1+a^2(\tilde{x}, \tilde{t})} B(\tilde{t}) - a(\tilde{x}, \tilde{t}) + \beta a(\tilde{x}, \tilde{t} - \Delta t) \right] \right\rangle_x
\end{aligned}
\tag{S3}$$

This dynamics of this reduced model is predominantly determined by the two parameters α and β . The parameter α indicates the relative strength of the actin-independent positive feedback, whereas the parameter β indicates the relative strength of the actin-mediated feedback loop. In the absence of actin ($\beta = 0$) the system breaks symmetry after a random initial condition, however the polar cap does not move (Supplemental Figure 3, lower panel; Supplemental Movie 4). In *rsr1Δ* cells in which the GAP activity is reduced or the Cdc24p activity is enhanced, the actin-mediated positive feedback dominates the negative loop ($\beta > 0$). The behavior of this strain is similar to cells lacking an actin cytoskeleton (Supplemental Figure 3, middle panel; Supplemental Movie 5). Only when the delayed actin-mediated loop is negative ($\beta < 0$) the polar caps starts move after symmetry breaking (Supplemental Figure 3, upper panel; Supplemental Movie 6).

Supplemental References:

Butty, A. C., Perrinjaquet, N., Petit, A., Jaquenoud, M., Segall, J. E., Hofmann, K., Zwahlen, C., and Peter, M. (2002). A positive feedback loop stabilizes the guanine-nucleotide exchange factor Cdc24 at sites of polarization. *Embo J* 21, 1565-1576.

Irazoqui, J. E., Gladfelter, A. S., and Lew, D. J. (2003). Scaffold-mediated symmetry breaking by Cdc42p. *Nat Cell Biol* 5, 1062-1070.

Shimada, Y., Wiget, P., Gulli, M. P., Bi, E., and Peter, M. (2004). The nucleotide exchange factor Cdc24p may be regulated by auto-inhibition. *Embo J* 23, 1051-1062.

Toenjes, K. A., Simpson, D., and Johnson, D. I. (2004). Separate membrane targeting and anchoring domains function in the localization of the *S. cerevisiae* Cdc24p guanine nucleotide exchange factor. *Curr Genet* 45, 257-264.

Wedlich-Soldner, R., Wai, S. C., Schmidt, T., and Li, R. (2004). Robust cell polarity is a dynamic state established by coupling transport and GTPase signaling. *J Cell Biol* 166, 889-900.

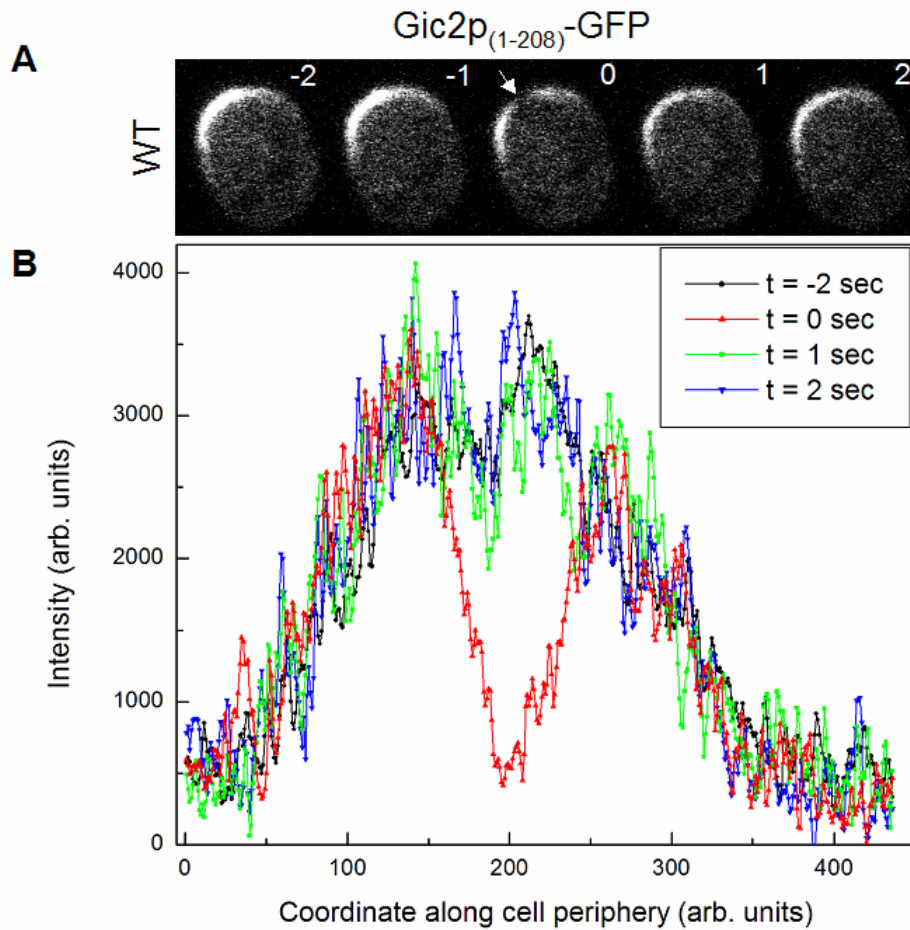


Figure S1. Recovery of the photobleached areas of the polar cap. (A) Gic2₍₁₋₂₀₈₎GFP is used as a reporter in a wild-type cell (strain ERT224.1). Time is given in seconds. The polar cap recovered to its initial fluorescence intensity in less than 1 second. (B) The intensity of the reporter is plotted along the cell periphery.

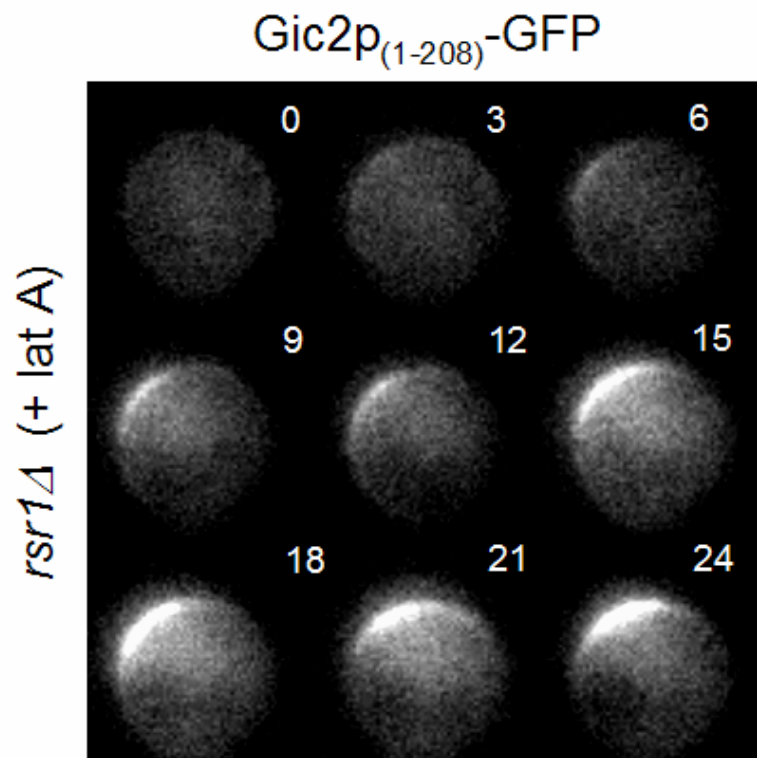


Figure S2. The effect of Lat-A on the establishment or maintenance of the polarized cap is shown for a cell in a *rsr1Δ* genetic background. $Gic2_{(1-208)}GFP$ is used as a reporter. In the presence of Lat-A, cells continue localizing the polar cap. However, the motility of the polar cap was significantly reduced. Time is reported in minutes.

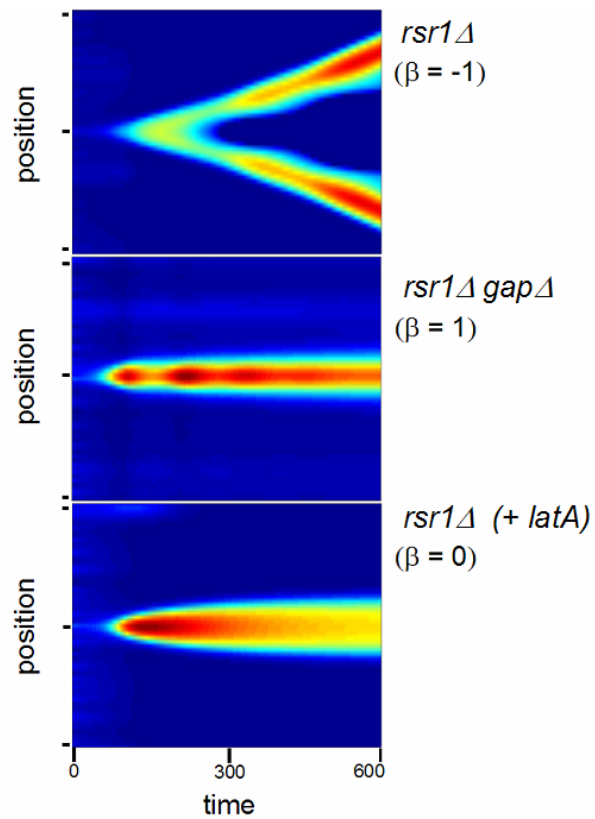


Figure S3. Numerical simulations of Eq. [3]. False color scale denotes the concentration of activated Cdc42p. The following parameters were used: $\alpha = 40$, $\Delta t = 500$, and $B(0) = 0.1$. A random initial condition for $a(\tilde{x}, 0)$ was chosen by random selecting a random number uniformly in the interval $[0, 0.3]$ for each position. Equation [S3] was numerically solved using MATLAB (Mathworks).

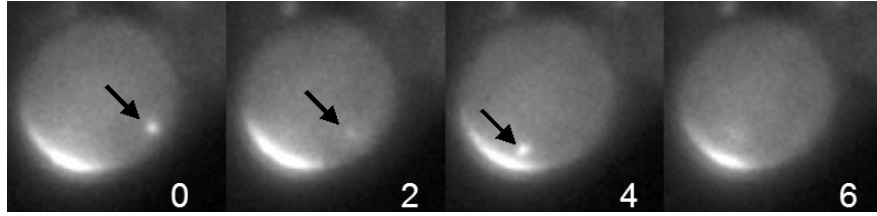


Figure S4. Motion of a $Gic2p_{(1-208)}$ -GFP labeled dot in a $rsr1\Delta$ strain in which $BEM3$ is overexpressed using the $GAL1$ promoter (strain ERT301.1). Cells were grown in synthetic media supplemented with 1% galactose.

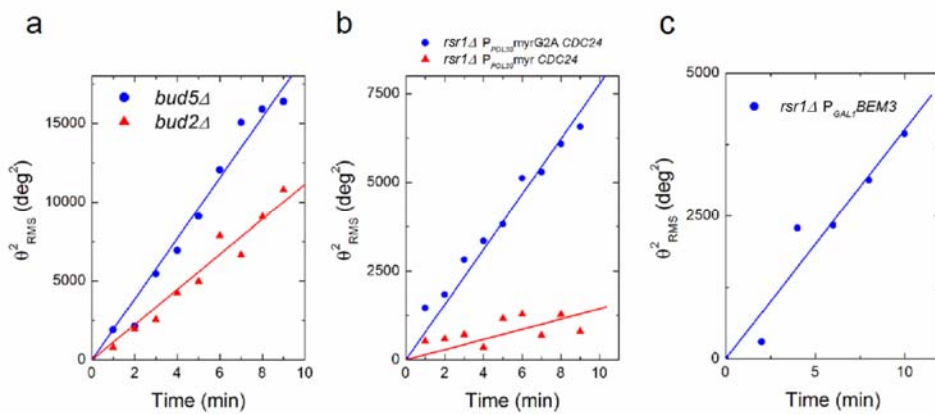


Figure S5. Θ^2_{RMS} is plotted a function of time with respect to the initial polarization angle at the start of the observation. Θ^2_{RMS} was obtained by averaging over at least ten different cells. The average slope, obtained by a linear least-square fit, of Θ^2_{RMS} -time relation defines the wave mobility. (a) The polar cap is highly motile in $bud2\Delta$ (strain ERT248.1, red triangles) and $bud5\Delta$ (strain ERT249.1, blue circles) cells. (b) The motility of the polar cap is damped when $CDC24$ is over-expressed and recruited to the cell membrane using a myristoylation-tag (strain ERT237.1, red triangles) compared to the control in which the myristoylation-tag is non-functional (strain ERT236.1, blue circles). (c) Polar cap motility in strain ERT303.1 in which $BEM3$ is overexpressed using the $GAL1$ promoter in a $\rho\sigma1\Delta$ background.

Table S1: Yeast strains used in this study:

Strain	Genotype
ERT224.1	BY4741; MATa; <i>MYO2::P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla-P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3</i>
ERT225.1	BY4741; Mat a; YGR152c(<i>rsr1</i>):: <i>kanMX4 MYO2::P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla-P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3</i>
ERT236.1	ERT225.1; <i>POL30::P_{POL30}-myr(G2A)-CDC24- T_{CDC24}-MET15</i>
ERT237.1	ERT225.1; <i>POL30::P_{POL30}-myr-CDC24- T_{CDC24}-MET15</i>
ERT248.1	BY4741; Mat a; YKL092c(<i>bud2</i>):: <i>kanMX4 Y04941 MYO2:: P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla- P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3</i>
ERT249.1	BY4741; MATa; YCR038C(<i>bud5</i>):: <i>kanMX4 MYO2:: P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla- P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3</i>
ERT250.1	BY4741; Mat a; YGR152c(<i>rsr1</i>):: <i>kanMX4 ::URA3-P_{ADHI}-GIC2:GFP CEN</i>
ERT254.1	BY4741; Mat a; YPL115c(<i>bem3</i>):: <i>kanMX4 MYO2:: P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla- P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3 RSR1::S.p.his5+</i>
ERT255.1	BY4741; Mat a; YOR127w(<i>rga1</i>):: <i>kanMX4 MYO2:: P_{MYO2}-rtTA- T_{CYCI}-LEU2 bla::bla- P_{tetO2}-GIC2(1-208):GFP- T_{CYCI}-URA3 RSR1::S.p.his5+</i>
ERT273.2	BY4741; Mat a; YGR152c(<i>rsr1</i>):: <i>kanMX4 BEM1/BEM1:CFP HIS</i>
ERT275.2	ERT225.1; <i>bla::bla-P_{GLN3}-RSR1- T_{RSR1}-MET15</i>
ERT276.3	ERT225.1; <i>IXR1::P_{IXR1}-RSR1- T_{RSR1}-MET15</i>
ERT282.3	ERT254.1 <i>RGAI::RGAI(1-60)-P_{POL30}-MET15</i>
ERT301.1	ERT225.1 <i>P_{BEM3}-BEM3:: P_{GALI}-BEM3-HIS3</i>

Table S2: Number of trajectories for each strain used to calculate the averaged square displacement. The duration of a trajectory is at least 10 minutes.

Strain	Number of trajectories
ERT224.1	24
ERT225.1 (-lat A)	39
ERT225.1 (+lat A)	32
ERT236.1	25
ERT237.1	25
ERT248.1	41
ERT249.1	27
ERT254.1	10
ERT255.1	33
ERT275.2	21
ERT276.3	57
ERT282.3	23
ERT301.1	12

