Symmetry breaking and the establishment of cell polarity in budding yeast: Supplementary Information

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Simulations were performed using MATLAB, on a model cell with 2D plasma membrane corresponding to a 5 μm diameter sphere (Figures 1 & 2) or 1D cell perimeter corresponding to a 5 μm diameter circle (Figure 3). All membrane-bound species have the same slow diffusion coefficient D_m and all cytosolic species have the same fast diffusion coefficient D_c. The cell dimensions, total Cdc42, total Bem1 complex, and (for Figures 2 & 3) total GDI are all constant. The ratio of membrane volume to cytoplasmic volume is indicated by η.

Model with Cdc42 and Bem1 complex:

For Figure 1, we model how the concentrations of the following five molecular species vary with time, as a result of reactions and diffusion:

GTP-Cdc42 (Cdc42T), GDP-Cdc42 (Cdc42D), Bem1 complex ($Bem1GEF_m$ on the membrane, $Bem1GEF_c$ in the cytoplasm), and Bem1 complex bound to GTP-Cdc42 ($Bem1GEF_mCdc42T$).

Model parameters are listed in Table S1. This model is described by the following equations (as in Goryachev and Pokhilko 2008 but without the GDI):

$$\frac{\partial Cdc42D}{\partial t} = D_m\nabla^2 Cdc42D - Cdc42D(k_2 Bem1GEF_m + k_{2p} Bem1GEF_m Cdc42T) + k_{2r} Cdc42T$$

$$\frac{\partial Cdc42T}{\partial t} = D_m\nabla^2 Cdc42T + Cdc42D(k_2 Bem1GEF_m + k_{2p} Bem1GEF_m Cdc42T) - k_{2r} Cdc42T - (k_4 Bem1GEF_c + k_5 Bem1GEF_m)Cdc42T + k_{5r} Bem1GEF_m Cdc42T$$

$$\frac{\partial Bem1GEF_m Cdc42T}{\partial t} = D_m\nabla^2 Bem1GEF_m Cdc42T + (k_4 Bem1GEF_c + k_5 Bem1GEF_m)Cdc42T - k_{5r} Bem1GEF_m Cdc42T$$

$$\frac{\partial Bem1GEF_c}{\partial t} = D_c\nabla^2 Bem1GEF_c + \eta(-k_3 Bem1GEF_c + k_{3r} Bem1GEF_m - k_4 Bem1GEF_c)Cdc42T$$

$$\frac{\partial Bem1GEF_m}{\partial t} = D_m\nabla^2 Bem1GEF_m + k_3 Bem1GEF_c - k_{3r} Bem1GEF_m - k_5 Bem1GEF_m \cdot Cdc42T + k_{5r} Bem1GEF_m Cdc42T$$

This model includes the following assumptions:

(i) Cdc42 is always on the membrane.

(ii) The Bem1 complex exchanges between membrane and cytoplasm. Both forms can bind GTP-Cdc42, and while bound the full complex stays on the membrane. Cdc42 does not undergo GTP hydrolysis while the Bem1 complex is bound.

(iii) Cdc42 GDP/GTP exchange is catalyzed by the Bem1 complex at the membrane. GEF-catalyzed exchange occurs by mass action (i.e. GEF activity never approaches saturation).

(iv) GAP activity is spatially uniform, and is incorporated in the first-order hydrolysis rate constant k_{2r}.

To successfully cluster GTP-Cdc42 by this mechanism, it is critical that total Bem1 complex abundance is low and membrane diffusion is slow. We modeled a scenario in which the GEF activity of the Bem1 complex is stimulated 20-fold upon binding to GTP-Cdc42, but this is not essential (increasing the basal GEF simply makes the cluster of GTP-Cdc42 larger).

Model with GDI as well as Cdc42 and Bem1 complex:

For Figures 2 & 3, we add the following three molecular species:

Free GDI in the cytoplasm (Ic), GDI bound to GDP-Cdc42 in the cytoplasm ($Cdc42I_c$), and GDI bound to GDP-Cdc42 on the membrane ($Cdc42I_m$).

Model parameters are listed in Table S1. This model is described by the following equations:

$$\frac{\partial Cdc42I_c}{\partial t} = D_c\nabla^2 Cdc42I_c + \eta(-k_1 Cdc42I_c + k_{1r} Cdc42I_m)$$

$$\frac{\partial Cdc42I_m}{\partial t} = D_m\nabla^2 Cdc42I_m + k_1 Cdc42I_c - k_{1r} Cdc42I_m + k_6 Cdc42D\cdot I_c - k_{6r} Cdc42I_m$$

$$\frac{\partial I_c}{\partial t} = D_c\nabla^2 I_c + \eta(-k_6 Cdc42\cdot I_c + k_{6r} Cdc42I_m)$$

$$\frac{\partial Cdc42D}{\partial t} = D_m\nabla^2 Cdc42D - k_6 Cdc42D\cdot I_c + k_{6r} Cdc42I_m - Cdc42D(k_2 Bem1GEF_m + k_{2p} Bem1GEF_m Cdc42T) + k_{2r} Cdc42T$$

$$\frac{\partial Cdc42T}{\partial t} = D_m\nabla^2 Cdc42T + Cdc42D(k_2 Bem1GEF_m + k_{2p} Bem1GEF_m Cdc42T) - k_{2r} Cdc42T - (k_4 Bem1GEF_c + k_5 Bem1GEF_m)Cdc42T + k_{5r} Bem1GEF_m Cdc42T$$

$$\frac{\partial Bem1GEF_m Cdc42T}{\partial t} = D_m\nabla^2 Bem1GEF_m Cdc42T + (k_4 Bem1GEF_c + k_5 Bem1GEF_m)Cdc42T - k_{5r} Bem1GEF_m Cdc42T$$

$$\frac{\partial Bem1GEF_c}{\partial t} = D_c\nabla^2 Bem1GEF_c + \eta(-k_3 Bem1GEF_c + k_{3r} Bem1GEF_m - k_4 Bem1GEF_c)Cdc42T$$

$$\frac{\partial Bem1GEF_m}{\partial t} = D_m\nabla^2 Bem1GEF_m + k_3 Bem1GEF_c - k_{3r} Bem1GEF_m - k_5 Bem1GEF_m\cdot Cdc42T + k_{5r} Bem1GEF_m Cdc42T$$

This model, which is identical to that of Goryachev and Pokhilko (2008) but with parameters adjusted according to Howell et al. (2009), includes the following additional assumptions:

(i) Cytoplasmic GDI can bind GDP-Cdc42 but not GTP-Cdc42, to generate an initially membrane-bound Cdc42-GDI complex that can then exchange between membrane and cytoplasm.

(ii) Cdc42 cannot exchange GDP for GTP while bound to GDI.

(iii) Membrane-bound Cdc42-GDI complex can dissociate to yield GDP-Cdc42p on the membrane and GDI in the cytoplasm.

The 1D simulations in Figure 3 are conducted on a line with periodic boundary condition, starting with Gaussian peaks of GTP-Cdc42. For competition, two peaks containing GTP-Cdc42 at a ratio of 55:45 were initiated at opposite ends of the cell (0.25L and 0.75L). For merging, two identical peaks were initiated at 0.25L and 0.625L.

Table S1: parameter values used in simulations

Parameter	**Figure 1**	**Figures 2 & 3**
k_1 (Cdc42-GDI complex: cytosol to membrane)	-	0.9 s^{-1}
k_{1r} (Cdc42-GDI complex: membrane to cytosol)	-	0.13 s^{-1}
k_6 (Cdc42-GDI association)	-	1.5 $\mu M^{-1}s^{-1}$
k_{6r} (Cdc42-GDI dissociation)	-	0.5 s^{-1}
k_2 (GEF activity of Bem1 complex)	0.2 $\mu M^{-1}s^{-1}$	0.16 $\mu M^{-1}s^{-1}$
k_{2p} (GEF activity of Bem1 complex bound to Cdc42)	10 $\mu M^{-1}s^{-1}$	0.35 $\mu M^{-1}s^{-1}$
k_{2r} (constitutive GAP activity)	5 s^{-1}	0.32 s^{-1}
k_3 (Bem1 complex: cytosol to membrane)	1 s^{-1}	10 s^{-1}
k_{3r} (Bem1 complex: membrane to cytosol)	1 s^{-1}	10 s^{-1}
k_4 and k_5 (Bem1 complex-Cdc42 association)	1 $\mu M^{-1}s^{-1}$	10 $\mu M^{-1}s^{-1}$
k_{5r} (Bem1 complex-Cdc42 dissociation)	2 s^{-1}	10 s^{-1}
η (ratio of membrane volume to cytoplasmic volume)	0.01	0.01
D_c (cytoplasmic diffusion)	10 $\mu m^2 s^{-1}$	10 $\mu m^2 s^{-1}$
D_m (membrane diffusion)	0.01 $\mu m^2 s^{-1}$	0.036 $\mu m^2 s^{-1}$
Total Cdc42 on membrane (Fig1)/ in a cell (Fig 2, 3)	25 μM	5 μM
Total Bem1 complex in a cell	0.0075 μM	0.017 μM
Total GDI in a cell	-	5 μM