

Table S1. Yeast strains used in this study

Strain	Background	Genotype	Source
DSY129	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ	Stone laboratory
RDY186	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ	Stone laboratory
XWY065	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ STE2-GFP-LEU2	This study
XWY086	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ STE2-GFP-LEU2, SLA1-RFP-URA	This study
XWY096	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-STE4-LEU2	This study
XWY097	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ STE2-GFP-LEU2, SEC3-RFP-URA	This study
XWY105	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-STE4-LEU2 SPA2-RFP-URA3	This study
XWY108	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ STE2-GFP-LEU2 SPA2-RFP-URA3	This study
XWY109	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-STE4-LEU2 STE2 ^{65A-7XR-GPAAD} -URA3	This study
XWY114	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-STE4-LEU2 STE2 ^{7XR-GPAAD} -URA3	This study
XWY117	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-STE4-LEU2 far1 ^{H7} -URA3	This study
XWY121	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ STE2-GFP-LEU2 bud1::URA3	This study
XWY137	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ SPA2-RFP-URA	This study
XWY147	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ GFP-CDC3-LEU2, SPA2-RFP-URA	This study
BSY008	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ SST2-GFP-KanMX6	This study
BSY031	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ SST2-GFP-KanMX6 SPA2-RFP-URA3	This study
BBY009	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ FAR1-GFP-KanMX6	This study
BBY015	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ FAR1-GFP-KanMX6 SPA2-RFP-URA3	This study
BBY019	BF264-15D	MAT α ade1 his2 leu2-3,112 trp1-1a ura3Δ far1 ^{H7} -GFP-KanMX6	This study

Table S2. Plasmids used in this study

Plasmid	Plasmid construction	Marker/type	Source
LHP1921	Ste2 ¹⁻⁴¹⁹ -GFP	LEU2/INT	Dunn et al., 2004
DSB156	pRS316-PSTE4-GFP-STE4 ^{1-1,272}	URA3/INT	Stone laboratory
DSB405	pRS406/RFP-BUD1	URA3/INT	Stone laboratory
XWB043	Yiplac211-Far1 ^{1,549-2,268}	URA3/INT	This study
XWB087	Yiplac211-SLA1 ^{2,491-3,732} -RFP	URA3/INT	This study
XWB097	Yiplac211-SEC3 ^{3,320-4,008} -RFP	URA3/INT	This study
XWB125	Yiplac128-Pcdc3-GFP-CDC3 ^{1-194, 196-400}	LEU2/INT	This study
MCB003	Yiplac128-PSTE4-GFP-STE4 ¹⁻⁵⁴⁷	LEU2/INT	This study
DLB3850	pRS306/STE2 ^{(600-1,296)-7XR-GPAAD-3'UTR}	URA3/INT	Lew laboratory

Table S3. Reaction formulae for the standard model (v.2)

Rx	Reaction	Comments
1	$\emptyset \xrightarrow{k_{rs}} II$	Synthesis of receptor dimers
2	$2k_{rl} \quad II + L \rightleftharpoons IA$	Association/dissociation of receptor and pheromone
3	$k_{rl} \quad IA + L \rightleftharpoons AA$	Association/dissociation of receptor and pheromone
4	$2k_{rlm} \quad \emptyset \xrightarrow{k_{gs}} G$	Synthesis of heterotrimeric G protein

5	$I + G \xrightarrow{k_{ga}} IA + Ga + Gbg$	Activation of G protein by partially liganded receptor
6	$AA + G \xrightarrow{2k_{ga}} AA + Ga + Gbg$	Activation of G protein by fully liganded receptor
7	$Ga \xrightarrow{k_{gad}} Gd$	Inactivation of G α
8	$Gd + Gbg \xrightarrow{k_{gd}} G$	Reassociation of G α and G $\beta\gamma$
9	$Yck + II + (G) \xrightarrow{k_{i0}} Yck$	Yck1/2-stimulated internalization of inactive receptor dimers and heterotrimeric G protein ^a
10	$Yck + IA + (G) \xrightarrow{k_{i1}} Yck$	Yck1/2-stimulated internalization of partially active receptor dimers and heterotrimeric G protein ^b
11	$Yck + AA \xrightarrow{2k_{i1}} Yck$	Yck1/2-stimulated internalization of fully active receptor dimers
12	$Gbg \rightleftharpoons GbgP$ k_{bp0}	Phosphorylation and dephosphorylation of G $\beta\gamma$
13	$Yck + GbgP \rightleftharpoons YckGbgP$ k_{yi}	Association/disassociation of G $\beta^P\gamma$ and Yck1/2
14	$GbgP + Fus3 \xrightarrow{k_{fa}} GbgP + Fus3A$	Activation of Fus3 by G $\beta^P\gamma$
15	$Fus3A \xrightarrow{k_{fd}} Fus3$	Deactivation of Fus3
16	$Fus3A + Gbg \xrightarrow{k_{bp1}} Fus3A + GbgP$	Phosphorylation of G β by active Fus3
17	$Ga + Fus3A + Gbg \xrightarrow{k_{bp2}} Ga + Fus3A + GbgP$	G α recruitment of active Fus3 to phosphorylate G β

^aThe stoichiometry of the internalized receptor dimer and heterotrimeric G protein is the ratio of their abundance.

Table S4. Reaction formulae for the monomeric-receptor variation of the model

Rx #	Reaction	Comments
1	$\emptyset \xrightarrow{k_{rs}} I$	Synthesis of receptor dimers
2	$I + L \rightleftharpoons A$ k_{rl}	Association/dissociation of receptor and pheromone
3	$\emptyset \xrightarrow{k_{gs}} G$	Synthesis of heterotrimeric G protein
4	$A + G \xrightarrow{k_{ga}} A + Ga + Gbg$	Activation of G protein by partially liganded receptor
5	$Ga \xrightarrow{k_{gad}} Gd$	Inactivation of G α
6	$Gd + Gbg \xrightarrow{k_{gd}} G$	Reassociation of G α and G $\beta\gamma$
7 cond1	$Yck + I \xrightarrow{k_{i0}} Yck$	Basal internalization of inactive receptor dimers

7	cond2	$\text{Yck} + I + \text{G} \xrightarrow{k_{i0}} \text{Yck}$	Basal internalization of inactive receptor dimers and heterotrimeric G protein
8		$\text{Yck} + A \xrightarrow{k_{i1}} \text{Yck}$	$\text{Yck1/2-stimulated internalization of active receptor dimers}$
9		k_{bp0}	Phosphorylation and dephosphorylation of $\text{G}\beta\gamma$
		$\text{Gbg} \rightleftharpoons \text{GbgP}$	
		k_{bpd}	
10		k_{yi}	Association/disassociation of $\text{G}\beta^P\gamma$ and Yck1/2
		$\text{Yck} + \text{GbgP} \rightleftharpoons \text{YckGbgP}$	
		k_{ya}	
11		$\text{GbgP} + \text{Fus3} \xrightarrow{k_{fa}} \text{GbgP} + \text{Fus3A}$	Activation of Fus3 by $\text{G}\beta^P\gamma$
12		$\text{Fus3A} \xrightarrow{k_{fd}} \text{Fus3}$	Deactivation of Fus3
13		$\text{Fus3A} + \text{Gbg} \xrightarrow{k_{bp1}} \text{Fus3A} + \text{GbgP}$	Phosphorylation of $\text{G}\beta$ by active Fus3
14		$\text{Ga} + \text{Fus3A} + \text{Gbg} \xrightarrow{k_{bp2}} \text{Ga} + \text{Fus3A} + \text{GbgP}$	$\text{G}\alpha$ recruitment of active Fus3 to phosphorylate $\text{G}\beta$

Table S5. Equations related to the spatial model

Eq.	Equation	Comments
1	$d = 4\pi r/n$	Surface distance between neighboring wedges
2	$[\text{L}]_i = \frac{[\text{L}]_f - [\text{L}]_b}{2} \cdot x_i + \frac{[\text{L}]_f + [\text{L}]_b}{2}$	The local pheromone concentration depends linearly on the x coordinate of the i th wedge x_i , and the pheromone concentration at the front of the cell $[\text{L}]_f$ and at the back of the cell $[\text{L}]_b$.
3	$D_S \nabla^2 [\text{S}]_i = \frac{[\text{S}]_{i-1} + [\text{S}]_{i+1} - 2[\text{S}]_i}{d^2}$	Lateral diffusion of the molecular species S in the i th wedge

Table S6. Equations of the reaction-diffusion system

Eq.	Equation
4	$\alpha = \min\left(1, \frac{[\text{G}]}{[\text{II}] + [\text{IA}] + [\text{AA}]}\right)$
5	$[\text{intII}] = 2k_{i0}[\text{Yck}][\text{II}]$
6	$[\text{intIA}] = k_{i1}[\text{Yck}][\text{IA}]$
7	$[\text{intAA}] = 2k_{i1}[\text{Yck}][\text{AA}]$
8	$\frac{d[\text{II}]}{dt} = D\nabla^2[\text{II}] + k_{rs}(i) - 2k_{rl}[\text{L}][\text{II}] + k_{rlm}[\text{IA}] - [\text{intII}]$
9	$\frac{d[\text{IA}]}{dt} = D\nabla^2[\text{IA}] + 2k_{rlm}[\text{L}][\text{II}] - k_{rlm}[\text{IA}] - k_{rl}[\text{L}][\text{IA}] + 2k_{rlm}[\text{AA}] - [\text{intIA}]$

$$\begin{aligned}
10 \quad & \frac{d[AA]}{dt} = D\nabla^2[AA] + k_{rl}[L][IA] - 2k_{rlm}[AA] - [intAA] \\
11 \quad & \frac{d[G]}{dt} = D\nabla^2[G] + k_{gs}(i) - 2k_{ga}[AA][G] - k_{ga}[IA][G] + k_{gd}[Gb][Gd] - \alpha[intII] - \alpha[intIA] \\
12 \quad & \frac{d[Ga]}{dt} = D\nabla^2[Ga] + 2k_{ga}[AA][G] + k_{ga}[IA][G] - k_{gad}[Ga] \\
13 \quad & \frac{d[Gd]}{dt} = D\nabla^2[Gd] + k_{gad}[Ga] - k_{gd}[Gb][Gd] \\
14 \quad & \frac{d[Gb]}{dt} = D\nabla^2[Gb] + 2k_{ga}[AA][G] + k_{ga}[IA][G] - k_{gd}[Gb][Gd] - k_{bp0}[Gb] + k_{bpd}[Gbp] - \\
& k_{bp1}[Fus3A][Gb] - k_{bp2}[Ga][Fus3A][Gb] \\
15 \quad & \frac{d[Gbp]}{dt} = D\nabla^2[Gbp] + k_{bp0}[Gb] - k_{bpd}[Gbp] + k_{bp1}[Fus3A][Gb] + k_{bp2}[Ga][Fus3A][Gb] - \\
& k_{yi}[Yck][Gbg] + k_{ya}[YckGbgP] \\
16 \quad & \frac{d[Fus3A]}{dt} = D\nabla^2[Fus3A] + k_{fa}[Fus3][GbgP] - k_{fd}[Fus3A] \\
17 \quad & \frac{d[Fus3]}{dt} = D\nabla^2[Fus3] - k_{fa}[Fus3][GbgP] + k_{fd}[Fus3A] \\
18 \quad & \frac{d[Yck]}{dt} = D\nabla^2[Yck] + k_{ya}[YckGbgP] - k_{yi}[Yck][GbgP] \\
19 \quad & \frac{d[YckGbgP]}{dt} = D\nabla^2[YckGbgP] - k_{ya}[YckGbgP] + k_{yi}[Yck][GbgP]
\end{aligned}$$

α is the ratio between receptor dimers and heterotrimeric G proteins. $[intII]$, $[intIA]$, and $[intAA]$ are the internalization rates of $[H]$, $[IA]$, and $[AA]$, respectively.

Table S7. Local delivery (synthesis) rates of receptor dimers and heterotrimeric G proteins

Eq.	Equation
20	$k_{rs_tot} = 2 \cdot n \cdot k_{rs_bas} \frac{S_R^\beta}{[totR]_{bas}^\beta + S_R^\beta}$
21	$k_{gs_tot} = 2 \cdot n \cdot k_{gs_bas} \frac{S_G^\beta}{[totG]_{bas}^\beta + S_G^\beta}$
22	$S_R = \max(2[totR]_{bas} - [totR]_{avg}, 0)$
23	$S_G = \max(2[totG]_{bas} - [totG]_{avg}, 0)$

$$24 \quad \left[\text{totR} \right]_{\text{avg}} = \frac{\int_i \left[\text{totR} \right]_i}{n}$$

$$25 \quad \left[\text{totG} \right]_{\text{avg}} = \frac{\int_i \left[\text{totG} \right]_i}{n}$$

$$26 \quad \left[\text{totR} \right]_i = \left[\text{II} \right]_i + \left[\text{IA} \right]_i + \left[\text{AA} \right]_i$$

$$27 \quad \left[\text{totG} \right]_i = \left[\text{G} \right]_i + \left[\text{Gbg} \right]_i + \left[\text{GbgP} \right]_i + \left[\text{YckGbgP} \right]_i$$

$$28 \quad k_{rs}(i) = k_{rs_tot} \frac{\left[\text{totR} \right]_i^\gamma}{\int_i \left[\text{totR} \right]_i^\gamma}$$

$$29 \quad k_{gs}(i) = k_{gs_tot} \frac{\left[\text{totG} \right]_i^\gamma}{\int_i \left[\text{totG} \right]_i^\gamma}$$

The total synthesis rates of receptor dimers and heterotrimeric G proteins are regulated by the total amounts of receptors and of G proteins, respectively. Their local delivery rates are regulated by the local amount of Gβγ. β determines how sensitive the total synthesis rate is to the total amount of the protein. γ determines how sensitive the local delivery rate is to the local amount of Gβγ.

Table S8. Variables and parameters

Parameter	Description	Initial value	Reaction rate ^a	Value
<i>r</i>	Cell radius	2 μm	krs	Depends on both global and local amounts of receptor dimers
<i>sa</i>	Cell surface area	50.27 μm ²	krl	3.32 × 10 ⁻³ μm ³ s ⁻¹ (Yi et al., 2003)
<i>v</i>	Cell volume	33.51 μm ³	krlm	0.01 s ⁻¹ (Yi et al., 2003)
<i>L(r)</i>	Pheromone concentration at the front of the cell	18 molecules/μm ³	kgs	Depends on both global and local amounts of G proteins
<i>L(−r)</i>	Pheromone concentration at the back of the cell	12 molecules/μm ³	kga	5.03 × 10 ⁻⁴ μm ² s ⁻¹ (Yi et al., 2003)
II	Inactive receptor dimer	Depends on location; estimated by fluorescence intensity	kgad	0.114 s ⁻¹ (Yi et al., 2003)
			kgd	50.3 μm ² s ⁻¹ (Yi et al., 2003)
II	Inactive receptor dimer	Depends on location; estimated by fluorescence intensity	ki0	1.01 × 10 ⁻⁵ μm ² s ⁻¹ (Yi et al., 2003)
IA	Partially active receptor dimer	0	ki1	5.05 × 10 ⁻⁵ μm ² s ⁻¹ (Jenness and Spatrick, 1986)
AA	Fully active receptor dimer	0	kbp0	2.90 × 10 ⁻⁴ s ⁻¹
G	Heterotrimeric G protein	Depends on location; estimated by fluorescence intensity	kbpd	2.50 × 10 ⁻⁴ s ⁻¹
Gα	Active Gα	0	kbp1	^b 1.00 × 10 ⁻⁵ μm ² s ⁻¹
Gδ	Inactive Gα	0	kbp2	^b 1.00 × 10 ⁻⁷ μm ² s ⁻¹
Gβγ	Gβγ	0	kyi	^b 5.00 × 10 ⁻³ μm ² s ⁻¹
GβγP	Gβγ	0	kya	^b 3.00 × 10 ⁻³ s ⁻¹
Yck	Yck1/2	4,000/sa (Ismael et al., 2016)	kfa	3.00 μm ² s ⁻¹ (Ismael et al., 2016)
YckGbgP	Yck1/2-Gβγ complex	0	kfd	1.00 s ⁻¹ (Maeder et al., 2007)
Fus3	Inactive Fus3	2,130/v (Ghaemmaghami et al., 2003)	k _{rs_bas}	0.159 μm ² s ⁻¹
Fus3A	Active Fus3	0	k _{gs_bas}	^c 3.98 × 10 ⁻² μm ² s ⁻¹
			β	2
			γ	2.5
			D	2.5 × 10 ⁻³ μm ² s ⁻¹ (Valdez-Taubas and Pelham, 2003)

^aValues for parameters listed in Tables S3, S4, S5, and S7.

^bValues used in computational model v.1 (Ismael et al., 2016).

^aThe basal rates of synthesis were selected to maintain the levels of receptor and G protein in vegetative cells, as reported in Ghaemmaghami et al. (2003).

Table S9. Sensitivity of model performance to the most critical unpublished parameters

Parameter	Standard value ^a	Total Gβ		Total receptor		Overlapping range ^d
		Polarity index ^b	Tracking rate ^c	Polarity index ^b	Tracking rate ^c	
K _{rs} (molecules/s)	0.16	0.080–0.80	0.0016–0.16	0.064–1.6	0.032–0.16	0.080–0.16
K _{gs} (molecules/s)	0.040	2.0 × 10 ⁻³ to 40	4.0 × 10 ⁻⁴ to 40	2.0 × 10 ⁻³ to 40	4.0 × 10 ⁻⁴ to 40	2.0 × 10 ⁻³ to 40
Receptor/Gβ	1.0	0.48–10	0.25–10	0.25–10	0.25–10	0.48–10
Synthesis control ^e	2.0	0.50–3.9	0.50–3.9	0.50–3.9	0.50–3.9	0.5–3.9
Biased secretion ^f	2.5	0.50–4.9	0.50–4.9	1.1–4.9	1.1–4.9	1.1–4.9

^aValues used in the standard model v.2.

^bCalculated by dividing the peak values of Gβ and the receptor by their average values at the tracking midpoint (halfway between the DS and CS). The range of parameter values that yielded polarity indices ≥ 1.1 are shown.

^cIndicates the range of parameter values that yielded Gβ and receptor tracking rates at least one-third that of the standard model.

^dThe range of parameter values that yielded functional tracking and polarization for both Gβ and the receptor, as defined above.

^eSynthesis control is the cooperative coefficient of regulated synthesis (β in Table S7).

^fBiased secretion is the cooperative coefficient of biased delivery (γ in Table S7).

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