**Yeast model of pheromone-induced cell polarity**

We used a model of yeast cell polarization induced by mating pheromone that has been previously described [1]. This model was based on the spatial dynamics of the heterotrimeric and Cdc42 G-protein cycles. Receptor (R) binds ligand (L) and becomes activated (RL). Activated receptor converts heterotrimeric G-protein (G) into activated -subunit (Ga) and free G (Gbg). All of these species are on the membrane. The connection between the two cycles is through free G which recruits cytoplasmic Cdc24 to the membrane. Membrane-bound Cdc24 (C24m) activates Cdc42. Activated Cdc42 (C42a) recruits the scaffold protein Bem1 (B1) to the membrane. Finally, a positive feedback loop is created because membrane-bound Bem1 can bind and recruit more Cdc24 to the membrane.

In the *bem1-299* mutant, *h* = 0 and . In the *bni1* mutant *ps* = 0.

 (S3-1)

 (S3-2)

 (S3-3)

 (S3-4)

 (S3-5)

 (S3-6)

 (S3-7)

 (S3-8)

 (S3-9)

 (S3-10)

 (S3-11)



# Polarized synthesis of receptor



**Conservation equations**



**Gradient input and cell dimensions**



**Table of Rate Constants**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter value** | **Reaction** | **Estimated range** | **Rationale** | **Ref** |
| *DR* | Diffusion or R | 0.001 | Travis FRAP expts |  |
| *DRL* | Diffusion or RL | 0.001 | Travis FRAP expts |  |
| *DG* | Diffusion or G | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DGa* | Diffusion or Ga | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DGbg* | Diffusion or Gbg | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DGd* | Diffusion or Gd | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DC24m* | Diffusion or C24m | 0.01 (0.005 – 0.02) | ??? |  |
| *DC42* | Diffusion or C42 | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DC42a* | Diffusion or C42a | 0.01 (0.005 – 0.02) | Travis FRAP expts |  |
| *DB1m* | Diffusion or B1m | 0.01 (0.005 – 0.02) | ??? |  |
|  |  |  |  |  |
|  | RL association |  | Measured | [2] |
|  | RL dissociation |  | Measured | [2] |
|  | R internalization |  | Fit to G-protein deactivation rate | [2] |
|  | R synthesis |  | Ensure steady-state receptor number of 10,000/cell | [2] |
|  | G-protein activation |  | Fit to G-protein activation and dose-response data | [2] |
|  | G-protein deactivation |  | Fit to G-protein activation and dose-response data | [2] |
|  | Heterotrimer association |  | Estimate (not rate-limiting) | [2] |
|  |  |  |  |  |
|  | Cdc42 deactivation | 0.02 – 2 s-1 | From literature; 10 fold-slower than heterotrimeric G-protein. Consistent with polarization time-course and *bni1* data | [3] |
|  | Cdc42 activation | 1e-3 – 1e-5 | To achieve proper steady-state levels of active Cdc42 | [3] |
|  | G recruitment of Cdc24 | 0.004 - 0.4 | Equivalent of *k20* in two-stage generic model; fit to produce proper polarization |  |
|  | Bem1 recruitment of Cdc24 | 3.3e-2 – 3.3e-4 | Equivalent of *k21* in two-stage generic model |  |
|  | Cdc24, membrane to cytoplasm | 0.1 - 1 | Ensures fast Cdc24 dynamics relative to Cdc42 |  |
|  | Bem1, membrane to cytoplasm | 0.01 - 1 | Same time-scale as Cdc42 deactivation rate |  |
|  | Bem1, cytoplasm to membrane | 1e-3 – 1e-5 | To achieve proper Bem1 polarization |  |
|  | Cla4 activation rate | 0.06 – 0.0006 | Establish proper ratio of active Cla4 |  |
|  | Cla4 deactivation | 0.1 – 0.001 | Same time-scale as positive feedback loop |  |
|  | Negative regulation of Cdc42 cycle | 0.1\*SA/3000 – 10\*SA/3000 | Negative feedback gain; ensures model stability/robustness |  |
|  |  |  |  |  |
| *q* = 100 | Hill coefficient for Gbgn\* | 1-100 |  |  |
| *h =8 or 0* | Hill coefficient for Bem1\* | 1-8 |  |  |
|  |  |  |  |  |
| *C24t* = 2000 mol | Total Cdc24 | 2,000 (1,000-3,000) |  |  |
| *B1t* = 3000 mol | Total Bem1 | 3,000 (2,000-5000) |  |  |
| *Rt* = 10,000 mol | Total receptor | 10,000 |  |  |
| *Gt* = 10,000 mol | Total G-protein | 10,000 |  |  |
| *C42t* = 10,000 mol | Total Cdc42 | 10,000 (5,000 – 20,000) |  |  |

The parameters can be divided into two groups: (1) Heterotrimeric G-protein parameters and (2) Cdc42 parameters. The first set of parameters was directly measured or was fit to G-protein activation/deactivation kinetics [2]. The time-scale was set by the G-protein deactivation rate. The Cdc42 dynamics were approximately 10-fold slower, and were dictated by the Cdc42 deactivation rate.

The Cdc24 and Bem1 dynamics formed the inner positive feedback loop and their parameters were tuned to ensure proper polarization of the model [1]. These constants represent the equivalent of *k20* and *k21* in the two-stage generic model.

The Cla4 dynamics represent in an approximate fashion the aggregate negative feedback regulation of Cdc42. This negative feedback loop was set to be on the same scale as the positive feedback loop, and the gain was adjusted to enhance model robustness.

The Hill constants *q* = 100 and *h* = 8 were the default values from the previous versions of the model [1]. Lower values i.e. *q* = 10 and *h* = 2 also produced polarization results similar to the default model.

Detailed individual parameter sensitivity analysis was not performed. Instead we scaled the two groups of parameters 10-fold up or 10-fold down to explore the effects of speeding up or slowing down the dynamics of each stage on the noise-resistance of the polarization (e.g. Fig. 3C).

**Initial conditions**

We may assume that [C42], [R], and [G] equally distributed along the surface with a total concentration and  respectively.



**REFERNCES**

1. Chou C-S, Nie Q, Yi T-M: **Modeling robustness tradeoffs in yeast cell polarization induced by spatial gradients.** *PLoS ONE* 2008, **3:**e3103.

2. Yi TM, Kitano H, Simon MI: **A quantitative characterization of the yeast heterotrimeric G protein cycle.** *Proc Natl Acad Sci U S A* 2003, **100:**10764-10769.