Signaling mechanisms that can yield Dose-Response Alignment

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Basic Sciences

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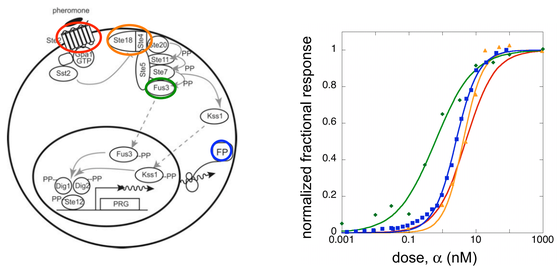
Introduction

The yeast pheromone response signaling system transmits information about the extracellular pheromone (e.g. α-factor) concentration to the cell nucleus. Experiments, from Brent's laboratory[1,2] and elsewhere[3], investigated the system response to pheromone at several "measurement points" in the signaling pathway (colored circles in cartoon). They found:

- The signaling system does not adapt over time, but functions at an essentially constant pace for >4 hours.
- Dose-response behaviors of the different measurement points are graded, meaning that the responses increase smoothly with increasing pheromone dose.
- Dose-response behaviors of the different measurement points are remarkably aligned. We call this phenomenon **DoRA,** for **Dose-Response Alignment**.

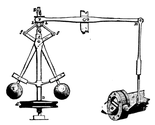


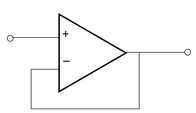
DoRA is widely observed in other systems too, likely because it improves information transmission[1].

What mechanisms enable signaling systems to exhibit DoRA? - this is the topic of this poster

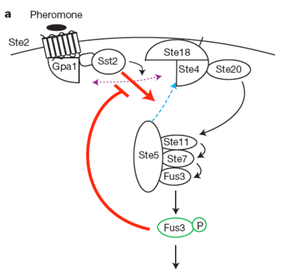
Negative feedback prediction

Negative feedback is often used in engineered systems to control downstream responses and to improve linearity between input and output. Examples range from steam engine governors to electronics operational amplifiers.



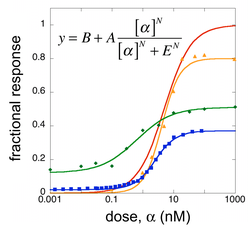


In prior work, several of us[1] discovered a new negative feedback in the yeast pheromone response system, from far downstream to far upstream (Fus3 to Ste5). This was a "smoking gun" for the negative feedback that creates DoRA. We planned to prove its feasibility using modeling.



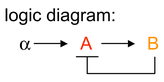
Methods

1. Rescale dose-response data so the y-axis gives the absolute fraction of protein that is activated, or fraction of maximal possible expression rate. These are best guesses, not hard numbers. Hill functions fit to these data became the inputs for further analysis.

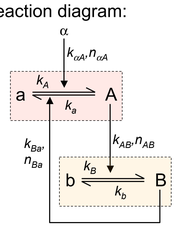


2. In the modeling scheme, nodes (e.g. A, below) are in equilibrium between inactive and active states (e.g. a and A). Arrows connect the nodes; the source of each arrow enzymatically activates or deactivates the destination node. The following model performs negative feedback.

logic diagram:



reaction diagram:



differential equations:

$$\frac{d[\mathrm{A}]}{dt} = [\mathrm{a}]\left(k_A + k_{\alpha A}[\alpha]^{n_{\alpha A}}\right) - [\mathrm{A}]\left(k_a + k_{Ba}[\mathrm{B}]^{n_{Ba}}\right)$$

$$\frac{d[\mathrm{B}]}{dt} = [\mathrm{b}]\left(k_B + k_{AB}[\mathrm{A}]^{n_{AB}}\right) - [\mathrm{B}]\left(k_b\right)$$

$$[\mathrm{a}] + [\mathrm{A}] = 1 \qquad [\mathrm{b}] + [\mathrm{B}] = 1$$

equilibrium constants:

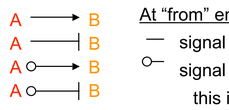
$$K_A = \frac{[\mathrm{A}]_{s.s.}}{[\mathrm{a}]_{s.s.}} = \frac{k_A + k_{\alpha A}[\alpha]^{n_{\alpha A}}}{k_a + k_{Ba}[\mathrm{B}]^{n_{Ba}}}$$

$$K_B = \frac{[\mathrm{B}]_{s.s.}}{[\mathrm{b}]_{s.s.}} = \frac{k_B + k_{AB}[\mathrm{A}]^{n_{AB}}}{k_b}$$

steady-state activities:

$$[\mathrm{A}]_{s.s.} = \frac{K_A}{1 + K_A}$$

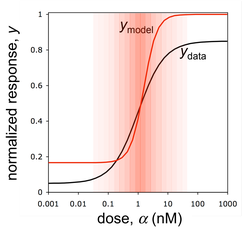
$$[\mathrm{B}]_{s.s.} = \frac{K_B}{1 + K_B}$$



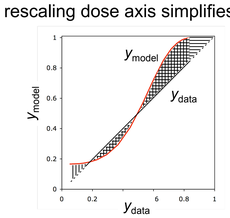
At "from" end (A)
- signal from "active" state
- signal from "inactive" state
- this is a "low-true" arrow

At "to" end (B)
- activate destination
- deactivate destination

3. Computationally optimize model parameters to get the best fit between model and experimental dose-responses. Fit errors are quantified with a new "slope-weighted RMS difference" metric.



rescaling dose axis simplifies result:



$$d = 100\sqrt{\int_{-\infty}^{\infty}(y_m - y_d)^2\left(c_d\left|\frac{dy_d}{d\alpha}\right| + c_m\left|\frac{dy_m}{d\alpha}\right|\right)d\alpha}$$

$$c_d = \frac{1}{2|y_d(\infty) - y_d(0)|} \quad c_m = \frac{1}{2|y_m(\infty) - y_m(0)|}$$

$$d = 100\sqrt{c_d\int_{y_d(0)}^{y_d(\infty)}(y_m - y_d)^2\,dy_d + c_m\int_{y_m(0)}^{y_m(\infty)}(y_m - y_d)^2\,dy_m}$$

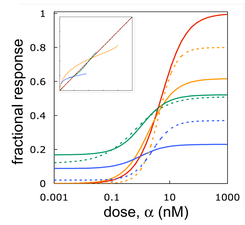
4. See what models can or cannot fit experimental data. The modeling scheme is *not* mechanistically accurate. Its results are meaningful if the model has the same capabilities and constraints as real signaling systems. Both model and reality can exhibit any Hill function, and are essentially limited to Hill functions, which suggests the model is valid.

Results

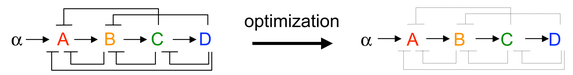
1. A linear network architecture fit the data poorly.

α → Receptor → G-protein → Fus3 → *PRM1*

Model dose-response amplitudes and EC_{50}s invariably decreased. This agreed with prior results[4].

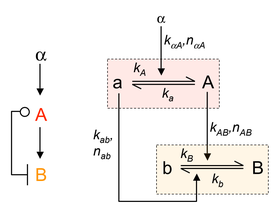


2. Negative feedback *never* improved fits, whether alone or in combination. Upon optimization, negative feedback arrow rates were always set to zero.

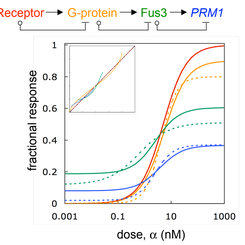


3. A survey of all possible two-node networks showed two mechanisms that can enable DoRA.
- A low-true negative feedforward (or low-true negative feedsame, which is similar), called "push-pull".
- Enzymatic cooperativity, in which the rate equation exponents could be ≠1. Shown with blue bars.

4. Push-pull mechanisms enable a substantially better fit. Here, the normal arrow activates the downstream node while the low-true arrow deactives it.



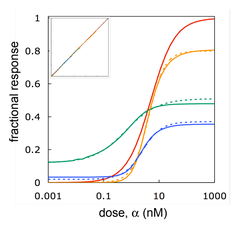
α → Receptor → G-protein → Fus3 → *PRM1*



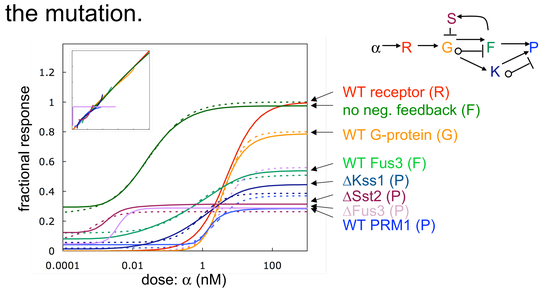
5. Enzymatic cooperativity enables a nearly perfect fit.

α → Receptor → G-protein → Fus3 → *PRM1*

parameter	value
$n_{\alpha,\mathrm{Receptor}}$	1.0
$n_{\mathrm{Receptor,G\text{-}protein}}$	2.3
$n_{\mathrm{G\text{-}protein,Fus3}}$	0.3
$n_{\mathrm{Fus3},PRM1}$	6.3

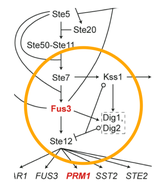


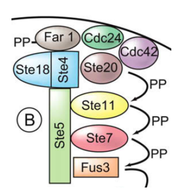
6. The model scheme, with push-pull mechanisms and cooperativity, fit 7 experimental yeast dose-response data sets well using a single set of parameters. An 8th data set did not fit, likely due to other consequences of the mutation.



Discussion

Push-pull mechanisms may arise in the yeast system from (*i*) parallel and complementary Fus3 and Kss1 pathways, (*ii*) a newly discovered G-protein activation mechanism[5].





Cooperativity may arise from (*i*) multiple phosphorylation in the kinase cascade, (*ii*) allosteric interactions in the Ste5 scaffold and other protein complexes.

Conclusions

The observed Dose-Response Alignment (DoRA) in the yeast pheromone response signaling system likely does *not* arise from negative feedback. Instead, it likely arises from novel push-pull mechanisms and/or cooperativity. These are biologically plausible.

References

1. Yu, Pesce, Colman-Lerner, Lok, Pincus, Serra, Holl, Benjamin, Gordon, and Brent, "Negative feedback that improves information transmission in yeast signaling" *Nature* 456:755-761, 2008.
2. Colman-Lerner, Gordon, Serra, Chin, Resnekov, Endy, Pesce, and Brent, "Regulated cell-to-cell variation in a cell-fate decision system" *Nature* 437, 699-706, 2005.
3. Yi, Kitano, and Simon, "A quantitative characterization of the yeast heterotrimeric G protein cycle" *Proc. Nat. Acad. Sci. USA* 100, 10764-10769, 2003.
4. Black and Leff, "Operational models of pharmacological agonism" *Proc. R. Soc. Lond. B* 220:141-162, 1983.
5. Bush, Colman-Lerner, In preparation, 2012.

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