Information for Appendix Table S1 from:

Single-cell profiling screen identifies microtubule-dependent reduction of variation in cell signaling

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Running title: Microtubule-dependent stabilization of signaling

High-throughput screen and follow up studies

Assembly of 96-format arrays of collection haploids isolated from single colonies

Selection of deletion strains included in the primary screen

Set 1: Unbiased Genes

This set consisted of 996 gene deletions randomly selected from the library. We made this set by picking clones from the library arrayed in 384 colonies format in order of appearance, starting in position A1, completing each row, and following with the next row. When a colony was missing we looked for a colony corresponding to the same gene deletion in the section for duplicates in the library, and added it if present. This selection was unbiased with respect to,

among other things: gene location in the genome, gene ontology, name, ORF number and any phenotype of the deletion strain.

Set 2: Kinases and Phosphatases

To assemble this set we searched the Saccharomyces Genome Database (SGD) for genes annotated with "viable systematic deletion phenotype" and with the "function" GO terms "protein kinase activity" and "phosphoprotein phosphatase activity". We retrieved 106 and 41 hits, respectively. Except for one gene in each set, all of these putative or confirmed protein kinases and phosphatases were represented in our modified gene deletion library. The total number of strains in this set was thus 145 (105 for protein kinases and 40 for phosphatases). Table S1 allStrains contains the list of all strains screened.

High-throughput growth, pheromone stimulation and flow cytometry measurements

We stored at 4^{o}C saturated cultures of 96-format clones from the modified deletion collection. To screen, we followed the procedure described below

Growth to exponential phase

We used a slotted pinning tool to inoculate 5µl of the saturated cultures stored at 4^{o}C into 500 µl of SDC media in 1.1 ml-capacity polypropylene 96-well plates. We grew these cultures to carbon exhaustion, 2 days at 30^{o}C. We then inoculated 5µl of the freshly saturated cultures into 250 µl of SDC in 300 µl-capacity polycarbonate 96-well plates, grew them for 8-10 h at 30^{o}C, and measured and saved the OD_{600} of all wells using a multiwell spectrophotometer. We used this measured OD information to calculate the dilution of inoculum needed to have most of the strains in exponential phase after 12-18 hours of growth. We then prepared several 300 µl-capacity polycarbonate plates as before and inoculated them using the 5 µl slotted pinning tool, at the calculated dilution, and 0.5, 1.5 and 3 times that amount.

Induction of the pheromone response system

After 15 h of growth, we measured OD600 of all plates and used the OD data to choose the plate in which the largest number of strains 1) was in exponential phase and 2) had enough cells to be used in the next step.

Immediately prior to stimulation, we sonicated the culture flat-bottom plates by "floating" the plates in the sonication bath of a S-3000 MP Misonix sonicator (Misonix Inc), set at power 10, for 2 minutes in two periods of 1 minute each with a 30 sec rest in between periods.

We next inoculated 5 µl of the sonicated cells into 250 µl of pheromone-containing medium in 300 µl-capacity polycarbonate 96-well plates. We followed the conditions described previously (Colman-Lerner et al., 2005). Briefly, stimulation media contained pheromone in SDC media containing 20 µg/ml caseine (SIGMA), to block pheromone binding to the plastic walls, 5-10 µM 1-NM-PP1, to inhibit Cdc28-as2 and 0.15 X strength PBS to buffer pH and thus prevent casein precipitation.

We incubated the cells in pheromone-containing medium for 3 h at 30 ºC for the screen and for most experiments, except when indicated. The 30 ºC incubation was done in an air heated ~30 cm rotation radius shaker at 250 rpm. After the end of the pheromone incubation period, we added 100 µg/ml cycloheximide to stop reporter accumulation and allow for complete fluorophore maturation. Yeast cells in cycloheximide retain their shape and external appearance for more than 10 hours at 30 ºC.

Finally, we sonicated the plates as described above and measured fluorescent protein expression by flow cytometry.

Flow cytometry measurements

We used a Becton-Dickinson LSRII flow cytometer equipped with a 100 mW 488 nm laser and a 150 mW 532 nm laser. All filters and dichroic mirrors were from Chroma. We used a threshold value of forward scatter (FSC) from the 488 nm laser to trigger data collection. We calibrated

threshold values of FSC to detect the smallest cells in an exponentially growing culture of wild type or mutant yeast cells. We measured YFP and mRFP (or mCherry) fluorescence from the light emitted during the 532 nm excitation, which was channeled, using mirrors, into an octagonal array of 8 photomultiplier tubes (PMTs), labeled A to H. Light entering the array was first split by a 735 nm long-pass dichroic mirror (735LP) and then split again by a 640 nm long-pass dichroic mirror (640LP). The light that came through the 640LP dichroic was filtered through a 675 nm band-pass filter of 50 nm wavelength width (675/50) before hitting the B PMT. The lower wavelength light that reflected from the 640 LP dichroic was directed towards a 600LP dichroic, and the reflected light from this was split by a 540LP dichroic. The light that passed the 540LP dichroic was filtered through a 550 nm band-pass of 10 nm width (550/10) before hitting the D PMT.

We took the signal from the B PMT as the fluorescence from mRFP (or mCherry). Cells expressing only YFP or CFP showed the same signal in this channel as wild type cells. We took the signal from PMT D as the fluorescence from YFP. Cells expressing only mRFP or CFP showed the same signal in this channel as wild type cells. We also took a signal for CFP, but this channel suffered from high background autofluorescence (largely from intracellular NAD(P)H UV-excited, cyan-emitting fluorescence) and was not useful for this project (we instead measured CFP expression by quantitative microscopy).

Derivation of formula for estimation of signal variation

Here we present the derivation of the formula we use for signal variation $\eta^2\left(P_y\right)$. In Colman-Lerner et al. 2005, we showed that the cell-to-cell variation observed in fluorescent reporter expression can be split into four contributions: (1) variation in signaling $\eta^2\left(P\right)$, (2) variation in gene expression capacity $\eta^2\left(G\right)$, (3) stochastic fluctuations in gene expression $\eta^2\left(\gamma_y\right)$, and a correlation term $2\rho\left(L,G\right)\eta\left(L\right)\eta\left(G\right)$, where $\rho\left(L,G\right)$is the correlation between mean signaling capacity and mean gene expression capacity, computed over the population. In a case where we used an inducible yellow reporter and a constitutive cyan reporter, labeling the corresponding quantities with subscripts *y* and *c*, we would thus have

$$\eta^2(y) = \eta^2(P_y) + \eta^2(G) + \eta^2(\gamma_y) + 2\rho(L_y, G)\eta(L_y)\eta(G)$$
$$\eta^2(c) = \eta^2(P_c) + \eta^2(G) + \eta^2(\gamma_c) + 2\rho(L_c, G)\eta(L_c)\eta(G)$$

The measured correlation between these two reporters can also be split into contributions from the two subsystems:

$$\rho(y,c) = \frac{\eta^2(G) + \rho(L_y, G)\eta(L_y)\eta(G) + \rho(L_c, G)\eta(L_c)\eta(G)}{\eta(y)\eta(c)} = \frac{\mathrm{cov}(y,c)}{\sigma_y \sigma_c}$$

In this work, we estimate $\eta^2(P_y)$ from the data in the following way

$$\eta^2(P_y) \approx \mathrm{var}\left(\frac{y}{\langle y \rangle} - \frac{c}{\langle c \rangle}\right)$$

where angle brackets indicate an average over the cell population of the enclosed quantity. Because the validity of this estimate may not be obvious at first, we derive it below:

Using the variance sum law, split the right-hand side of the previous equation into

$$\mathrm{var}\left(\frac{y}{\langle y \rangle} - \frac{c}{\langle c \rangle}\right) = \mathrm{var}\left(\frac{y}{\langle y \rangle}\right) + \mathrm{var}\left(\frac{c}{\langle c \rangle}\right) - 2\,\mathrm{cov}\left(\frac{y}{\langle y \rangle}, \frac{c}{\langle c \rangle}\right)$$

From the definition of η^2, and properties of covariance, this can be re-written as

$$\mathrm{var}\left(\frac{y}{\langle y \rangle} - \frac{c}{\langle c \rangle}\right) = \eta^2(y) + \eta^2(c) - \frac{2\,\mathrm{cov}(y,c)}{\langle y \rangle \langle c \rangle}$$

Using the definition of η, we can say

$$\mathrm{var}\left(\frac{y}{\langle y\rangle}-\frac{c}{\langle c\rangle}\right)=\eta^2(y)+\eta^2(c)-\frac{2\rho(y,c)\sigma_y\sigma_c}{\langle y\rangle\langle c\rangle}=\eta^2(y)+\eta^2(c)-2\rho(y,c)\eta(y)\eta(c)$$

Insert the definitions from Colman-Lerner et al 2005, as above, and perform the cancellations:

$$\mathrm{var}\left(\frac{y}{\langle y\rangle}-\frac{c}{\langle c\rangle}\right)=\eta^2(y)+\eta^2(c)-2\left(\eta^2(G)+\rho(L_y,G)\eta(L_y)\eta(G)+\rho(L_c,G)\eta(L_c)\eta(G)\right)$$

$$\begin{aligned}\mathrm{var}\left(\frac{y}{\langle y\rangle}-\frac{c}{\langle c\rangle}\right)&=\eta^2(P_y)+\eta^2(G)+\eta^2(\gamma_y)+2\rho(L_y,G)\eta(L_y)\eta(G)\\&\quad+\eta^2(P_c)+\eta^2(G)+\eta^2(\gamma_c)+2\rho(L_c,G)\eta(L_c)\eta(G)\\&\quad-2\left(\eta^2(G)+\rho(L_y,G)\eta(L_y)\eta(G)+\rho(L_c,G)\eta(L_c)\eta(G)\right)\end{aligned}$$

$$\mathrm{var}\left(\frac{y}{\langle y\rangle}-\frac{c}{\langle c\rangle}\right)=\eta^2(P_y)+\eta^2(\gamma_y)+\eta^2(P_c)+\eta^2(\gamma_c)$$

Since the constitutive pathway variation and the gene expression noise terms, i.e. the last three terms on the right-hand side, are all small (see supplement to Colman-Lerner et al 2005), this is a good way to estimate the inducible pathway variation $\eta^2(P_y)$.

The neglected terms are all positive, thus the computed quantity (the left-hand side) represents an upper limit for $\eta^2(P_y)$, i.e.

$$\mathrm{var}\left(\frac{y}{\langle y\rangle}-\frac{c}{\langle c\rangle}\right)\hat{a}\ \eta^2(P_y)$$

To summarize, *y* and *c* are values of the total fluorescence (two different colors) from each cell in an isogenic population. The variance of the difference between *y* and *c*, both normalized by their respective means, is a measure of the uncorrelated variation visible on a scatter plot of *y* vs. *c*. If both *y* and *c* are reading constitutive promoters, then this is in turn a measure of $\eta^2(\gamma_y)+\eta^2(\gamma_c)+\eta^2(P_c)$, i.e. stochastic noise in gene expression. If, on the other hand, y is

reading an induced promoter and *c* a constitutive promoter, then we know from previous work (Colman-Lerner et al., 2005) that the variance of the difference between the normalized fluorescences includes a much larger contribution from $\eta^2\left(P_y\right)$. We can thus use this variance as an estimate of the cell-to-cell variation in transmitted signal, $\eta^2\left(P_y\right)$.

Appendix Table S1 legend

Raw reporter gene values for strains screened in this work. Table shows gene name, plate number in the diploid variation collection, and raw values for fluorescent signals at low and high pheromone doses. It also shows normalized variation in each reporter signal and η2(P) for each strain calculated as above