

A single-cell-based genetic screen identifies gene mutations that alter novel axes of quantitative system behavior in the pheromone pathway of *Saccharomyces cerevisiae*.

C. Gustavo Pesce*, Alejandro Colman-Lerner, Daniel J. Rockwell, Rich Yu and Roger Brent

Molecular Sciences Institute, Berkeley, California, USA

*gpsce@molsci.org

Conversion of graded extracellular information into graded cellular outputs by multi-component signaling pathways is a ubiquitous yet mysterious process: How is variability controlled? How is the graded information preserved through a signaling cascade? What determines the dynamic range? To gain insights into these problems we isolated *S.cerevisiae* mutants with altered quantitative behavior in their response to mating pheromone. We identified mutations with specific effects on the sensitivity, variability, dynamic range, amplitude and/or degree of graded behavior of the response. Often, available information on the mutated gene suggested testable mechanistic hypotheses about the underlying molecular mechanisms that cause these changes.

To quantify pheromone system output in single cells we used a pheromone inducible promoter (P_{PRM1}) driving the expression of the red fluorescent protein mRFP and a second, pheromone insensitive promoter (P_{ACT1} or P_{BMH2}) driving the expression of the yellow fluorescent protein YFP. The ratio between the red and yellow intensities is a measure of the output of the pheromone pathway, independent of the cell's global gene expression output [1]. We also eliminated four sources of distortion: the protease Bar1, the activity of Cdc28, cell division and fluorescent protein maturation [1]. We measured six quantitative aspects of pathway performance: mean values of total output (Y), pathway output (L), expression output (G) and variability in each of them ($\eta^2(Y)$, $\eta^2(P)$ and $\eta^2(G)$). As shown in figure 1, wild type yeast cells display a characteristic set, a signature, of dose-responses for all these quantities.

Figure 2 shows examples of mutants. Panel A shows that cells lacking either of the two partially "redundant" effector MAP kinases, Fus3 or Kss1, display a shift in EC_{50} (the dose that yields half-maximal pathway activity) to lower values (more sensitive) and a reduction in $\eta^2(P)$. Panel B shows 3 examples of a larger group of genes encoding ribosomal proteins that have a common, specific phenotype: reduced G (as expected), and increased L . Feedbacks like this provide important homeostatic control to signaling pathways.

Panel C shows that cells lacking the transcription factor Kar4, required for induced expression of the motor protein Kar3, show a dramatically compressed dynamic range of L that nonetheless displays a nearly normal EC_{50} value. Thus, the dynamic range can be modified independently of other system quantities. Kar3 is required to move the nucleus towards the shmoo tip where pheromone receptors cluster. Cells lacking Kar3 and other proteins involved in nuclear positioning display consistent alterations in pheromone signaling, which suggested to us the novel hypothesis that nuclear positioning determines signaling output.

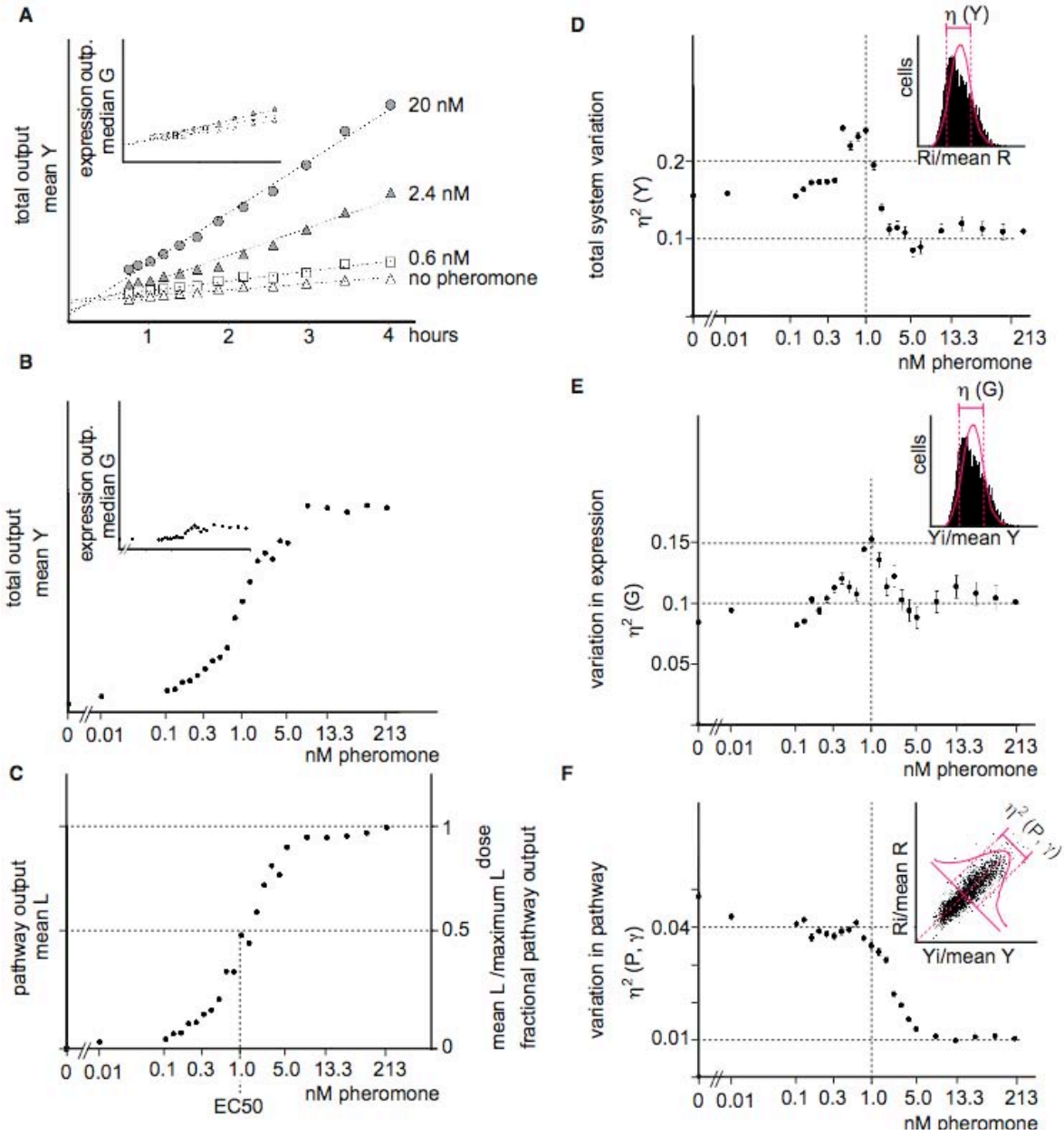


Figure 1: Quantitative behavior of the pheromone response system. a) time dependent increase in the mean mRFP (main) and YFP (inset) in cells continually exposed to the pheromone dose indicated; (b-f) Plots show changes in the following system quantities vs pheromone dose (cells were exposed to pheromone for 3 h): b) main: total system output or mean Y (mRFP); inset: gene expression output or mean G (YFP) c) left axis: pathway output or mean L (mRFP/YFP), right axis: normalized L (fraction of maximum) d) total variation in system output ($\eta^2(Y)$), variance in mRFP/ mean mRFP squared; e) total variation in gene expression output ($\eta^2(G)$), variance in YFP/ mean YFP squared; f) cell-to-cell variation in pathway output (plus noise in gene expression) ($\eta^2(P, \gamma)$), variance of the spread across the mRFP vs YFP correlation line. Insets in (d-f): schematic representation of the quantity plotted.

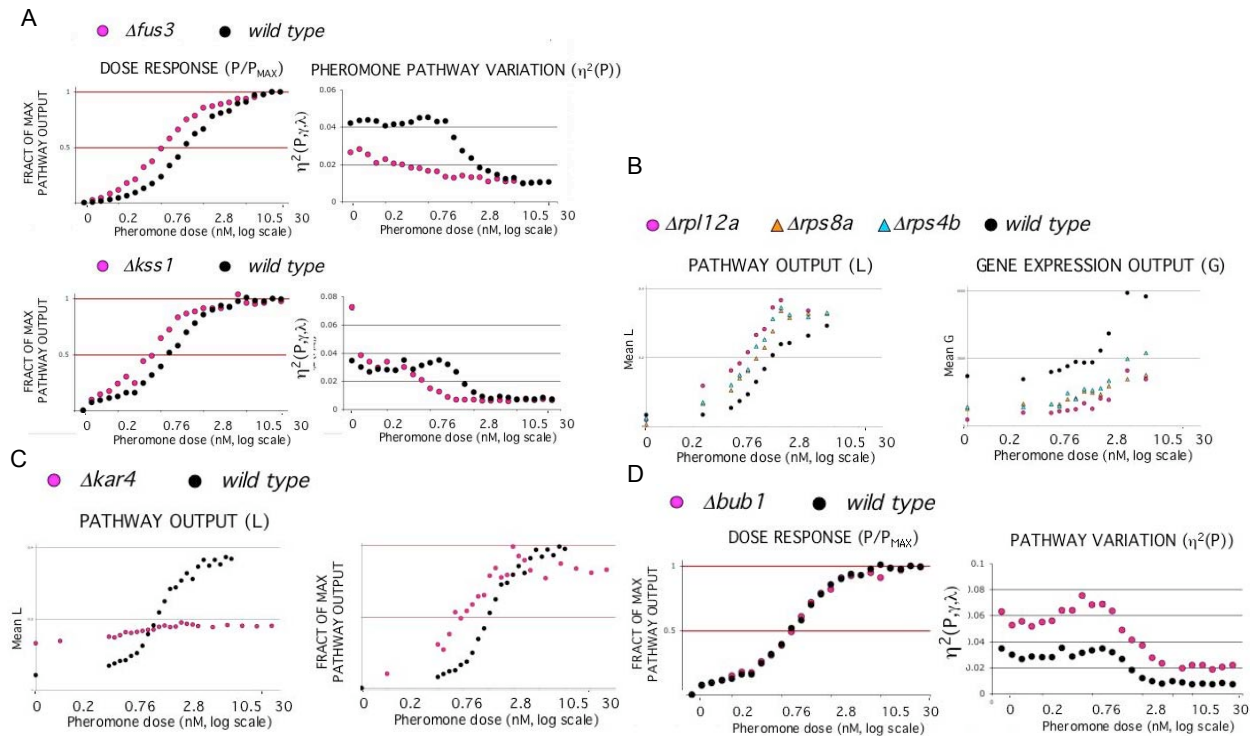


Figure 2: Gene deletions that alter quantitative system behavior. Plots show changes in the following system quantities vs pheromone dose: a) top plots: $\Delta fus3$ and wild type, lower plots: $\Delta kss1$ and wild type; left: normalized pathway output, right: cell-to-cell variation in pathway output; b) $\Delta rpl12$, $\Delta rps8a$, $\Delta rps4b$ and wild type; left: pathway output, right: gene expression output; c) $\Delta kar4$ and wild type; left: pathway output, right: normalized pathway output; d) $\Delta bub1$ and wild type; left: normalized pathway output, right: cell-to-cell variation in pathway output.

Last, we show in Panel D that cells lacking the protein kinase Bub1 have an increased $\eta^2(P)$ at all doses and a perfectly normal dose response in L. This again defines an independently regulated quantity and a new function for Bub1, apparently unrelated to its role in the spindle assembly checkpoint.

The identification of genes required for the precise maintenance of the quantities that define the signaling output of the mating pathway supports the view that the system has evolved to modulate these quantities, plausibly in response to selective pressure for an increased capacity to transmit information about dose. These results also imply that measurements of quantitative outputs may be a broadly applicable approach to identify new regulatory mechanisms, assign new roles to gene products and gain a deeper understanding of cell behavior.

References

[1] Colman-Lerner, A., *et al.* (2005) Regulated cell-to-cell variation in a cell-fate decision system. *Nature* 437, 699-706.