Cell

The Least Mating Pathway: Synthetically Refactoring a Familiar Signaling System for New Applications

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https://doi.org/10.1016/j.cell.2019.04.003

Synthetic refactoring makes naturally occurring regulatory systems more amenable to manipulation by removing or recoding their natural genetic complexity. Shaw et al. apply this technique to the yeast mating response pathway, creating a simplified, highly engineerable signaling module that can be used to construct precisely optimized, application-specific GPCR biosensors.

The intrinsic regulatory complexity of multi-gene systems frequently thwarts their harnessing for productive application. For even apparently simple systems, non-additive, pleiotropic, or even hidden regulatory interactions between genes or cellular components make rational tuning and rewiring extremely unpredictable. "Refactoring"—a term borrowed from software design—describes a synthetic biology approach that mitigates these complications by physically abstracting the genes encoding a system away from their native genomic context (Smanski et al., 2016). Operationally, this involves knocking out the native copies, and then reintroducing synthetically recoded versions that are placed under user-defined regulatory control. Refactoring a multigene system makes it more engineerable; it decouples the system from confounding native regulation, while the modularity it imposes can facilitate fine-tuning of component expression as well as connection to new inputs and outputs. In this issue of *Cell*, Shaw et al. (2019) refactor the yeast mating response pathway, recomposing its core G protein-coupled receptor (GPCR)/MAP kinase (MAPK) signaling cascade to create a broadly applicable platform for cell-based biosensing.

Few regulatory systems have been as exhaustively characterized as the mating pathway, which mediates the signaling response to exogenous pheromone during haploid mating of budding yeast cells. The pathway features a heterotrimeric GPCR linked to a canonical MAPK cascade. Activation of the signaling cascade reorganizes much of the cell's physiology, resulting in cell cycle arrest, activation of an extensive transcriptional program, and cytoskeletal reorganization that prepares cells for fusion with a nearby partner (Figure 1) (Bardwell, 2005). Study of the pathway has played a leading-edge role during the emergence of several fields of basic research. This includes systems biology, where the mating pathway provided a model for quantitative study of the dynamics, information transfer, and noise propagation in signaling networks (Atay and Skotheim, 2017). Thanks to comprehensive cataloging of its components and their interactions, the mating pathway presented a ready testbed for exploring the consequences of regulatory rewiring (Bashor et al., 2008; Galloway et al., 2013; Gordley et al., 2016; Park et al., 2003).

Shaw et al. have extended this run of groundbreaking mating pathway engineering by using a refactoring approach to create a tunable, highly composable version of the core GPCR/MAPK signaling pathway (Figure 1). To accomplish this, the authors used CRISPR-mediated genome engineering to first knock out a total of 18 genes, including those encoding the GPCR and G protein subunits. Additionally, they eliminated genes involved in both downstream transcriptional response (*STE12*) and pathway feedback (*SST2*, *BAR1*) and cross-talk (*FAR1*), simplifying core pathway signaling function and effectively decoupling it from the broader mating response network. Left intact were the five genes comprising the MAPK cascade (*STE5*, *STE20*, *STE11*, *STE7*, *FUS3*), which the authors effectively treat as a discreet "black box" module to which inputs and outputs can be connected in plug-and-play fashion.

To create an initial benchmark for refactored pathway behavior, the authors reintroduced the minimum set of genes necessary to mediate pheromone response: the GPCR module—the receptor (*STE2*) and the Gα, β, and γ subunits (respectively *GPA1*, *STE4*, and *STE18*)—and the gene for Ste12, the transcription factor (TF) responsible for mating-responsive gene activation. The resulting system, termed "Quasi-WT," reconstituted pheromone signaling as measured by mating-induced GFP transcriptional reporter, exhibiting a highly sensitized dose response due to removal of negative feedback pathways. To explore the interplay between component expression and refactored pathway behavior, the authors used a promoter library to systematically tune GPCR and G protein subunit expression levels. Consistent with computational predictions used to motivate their work, they found a balance of the three subunit proteins to be key for maximizing the pathway's fold-change response. The response could be further boosted by engineering a synthetic, MAPK-activated TF and reporter that replaces Ste12, fully decoupling the refactored pathway from mating-responsive transcriptional activation.

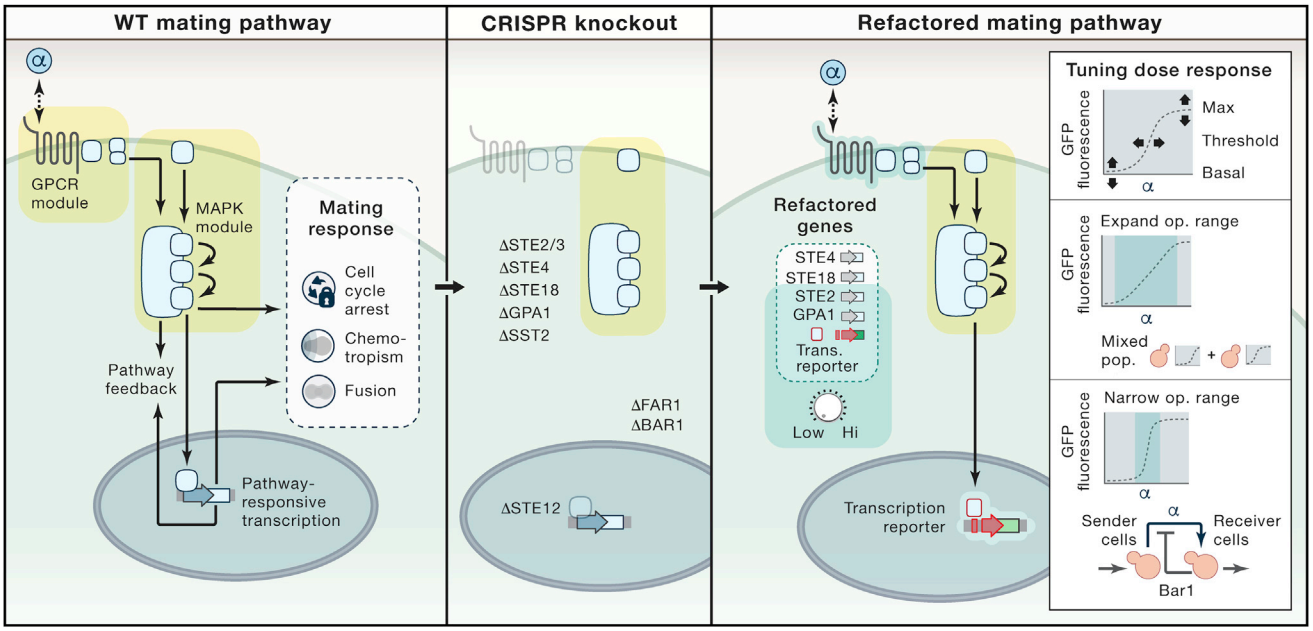

Figure 1. Steps Outlining a Synthetic Refactoring of the Yeast Pheromone Response Pathway

Shaw et al. refactor the yeast mating pathway via CRISPR gene knockout, abolishing transcriptional activation, cell cycle arrest, and pathway feedback, effectively disengaging the core signaling cascade from the cellular outputs that drive mating response. Adding back components of that comprise the GPCR cycle, and by directing MAPK module output to a synthetic transcriptional reporter, they are able to create a system in which quantitative features of pathway dose response can be predictively tuned.

Based on their analysis, the authors identified expression levels of the receptor and Gα subunits, as well as the output of the TF reporter, as "knobs" that could be used to tune through dose-response space, permitting comprehensive control over basal and maximal output, as well as activation sensitivity. To obtain control over dose-response cooperativity, the authors turned to engineered consortia, demonstrating the ability to combine different strains to adjust the operational range of the pathway response. Dose-response broadening (less cooperative) was achieved by mixing strains with different response sensitivities, while narrowing (more cooperative) involved engineering a sender-receiver system featuring feedback-mediated coupling between an "amplifier" strain that sense ligand and produces pheromone and a "reporter" strain that both senses, reports on, and degrades the signal. These demonstrations points to clear applications for development of biosensor applications. The refactored pathway can be coupled to a wide variety of heterologous GPCR inputs to detect high-value chemical inputs, while the dose-response programming framework offers the ability to optimizing sensing and interpolate between linear-sensor or switch-like actuator function depending upon the application.

While synthetic refactoring has been contextualized as a mostly application-driven activity, work by Shaw et al. hints at the power of this approach for answering fundamental questions about systems-level behavior. From this perspective, the emerging synthetic biology toolkit—replete with ever-advancing DNA manipulation and genome-engineering techniques—can be viewed as a radically advanced form of reverse genetics that can be used to explore connections between the modularity, evolvability, and engineerability of regulatory networks (Mukherji and van Oudenaarden, 2009). Indeed, Shaw et al. were able to validate a number of important hypotheses regarding the behavior of the GPCR module, including demonstrating dependence of GPCR sensing function on component stoichiometry rather than intrinsic GPCR properties. Given the ever-decreasing cost of gene synthesis, synthetic refactoring will likely be used to systematically perturb and study regulatory systems of increasing size and complexity (Bashor and Collins, 2018). For example, in mammalian systems, studying synthetic chromatin loci containing genes that have been refactored to eliminate splice variation, or to evade chromatin regulation, could provide opportunities to decouple multi-scale chromatin regulation of metazoan genes from the systems-level function of their products.

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