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Systems Biology: A Switch for Sex

The yeast pheromone response pathway works in a graded fashion, such that more pheromone leads to more response, but a recent study has shown that small modifications convert it into a bistable switch, with implications for the evolution and engineering of reaction networks.

Steven S. Andrews[1] and Adam P. Arkin[1,2]

Feedback is the route to the creation of complex, yet controlled, function in nearly all engineered systems. It is at the heart of stable switches and oscillators, central to the stabilization of even continuous behaviors under uncertain environmental conditions, and essential for the coordination of multiple systems. Feedback, well deployed, leads to predictable function, robust behavior and controllable outcomes. Errors in feedback design can be catastrophic. It is not surprising, then, that cellular networks have evolved to be rife with feedback circuits. But there may be more or less 'evolvable' ways to achieve these feedbacks. Evolvability is the ease with which a system can gradually change function under selection, without having to transit through unfit behaviors [1,2]. If biological systems are evolvable, and there is evidence that they are, then this has profound implications for the dynamics of evolution and the feasibility of emerging engineering disciplines like synthetic biology. In a study published recently in *Current Biology*, Ingolia and Murray [3] demonstrate the feedback 'flexibility' of the *Saccharomyces cerevisiae* pheromone response system: the authors introduced relatively simple changes to the circuit which converted it from a graded-response system to a switching system. The ease of these changes, and the behaviors derived, shed light on the evolvability and engineerability of cellular networks.

S. cerevisiae, bakers yeast, generally lives as a diploid organism. However, individuals can also be haploid in either of two types, called *MAT***a** and *MAT*α. Mating between these two types, which forms a fused diploid cell, is initiated during reciprocal communication between *MAT*α cells and *MAT***a** cells when they release pheromones called α-factor and **a**-factor, respectively, each sensed by the complementary mating type. The α-factor branch of this interaction is easier to explore experimentally, so it is the best studied. After α-factor is sensed by cell-surface receptors, the signal is transduced through many steps that eventually result in the activation of so-called pheromone response genes [4] (Figure 1). The products of these genes initiate the mating response through cell shape changes, arrested cell division, and other processes.

In wild-type cells, the gene-expression response to α-factor is graded, meaning that the amount of expression increases gradually with increasing amounts of pheromone. It is also reversible, such that gene expression increases when pheromone is added, and decreases when pheromone is removed. Thus, despite the many steps in the

signaling pathway, yeast signal transduction functions as a telephone: the signal that comes out is essentially the same as the signal that went in; this is supported by unpublished results of Roger Brent's group (personal communication) and suggested by prior observations [5,6]. The signal may be modified at various stages in the transmission, but is corrected by the end of the pathway so that the output mimics the input. Other parties can, and do, listen in on the transmitted signal.

Ingolia and Murray [3] created a positive feedback in the response pathway by fusing dominant active alleles of upstream signaling proteins, such as the Ste11 MAP kinase kinase kinase, to a strongly pheromone-induced promoter, P_{FUS1} (Figure 1). In a number of the constructed cells, the output is abruptly turned on by addition of α-factor, and then stays on even after α-factor is removed. Other mutants, after being activated by α-factor, showed a slow spontaneous switching from 'on' to 'off' which was sped up using a chemical called Shokat's inhibitor. Once gene expression was switched off, it remained off. For the most part, these feedbacks had no effect before the cells were exposed to α-factor, and little effect during exposure, but then maintained pheromone response activation after α-factor was removed. Interestingly, for some of the feedback systems and appropriate input conditions, controlled diversity was observed in which some cells were fully 'on' and some fully 'off'. This is significant because such diversification has recently been recognized to have possible evolutionary benefit [7,8].

Negative feedback usually stabilizes systems and decreases their sensitivity to noise; negative feedback is integral to homeostasis. In contrast, positive feedback generally drives systems away from intermediate output values and toward outputs that are either fully turned off or fully turned on. This makes positive feedback useful for biochemical circuits that direct cells towards discrete states. These include the lysis–lysogeny states of λ phage, the states of the eukaryotic cell cycle and cell differentiation [9]. Depending on the network architecture and reaction rate constants, positive feedback can lead to one-way switches, often found in cell fate decisions [10], toggle switches, as in cell-cycle control networks [11], or just to amplification of stochastic fluctuations [12]. There can even be single kinetic parameters that can switch a given system from one of these modes to the other [9]. Ingolia and Murray [3] demonstrate some of these behaviors with their various constructs which demonstrates the ease with which rewiring can create a spectrum of qualitatively different behaviors.

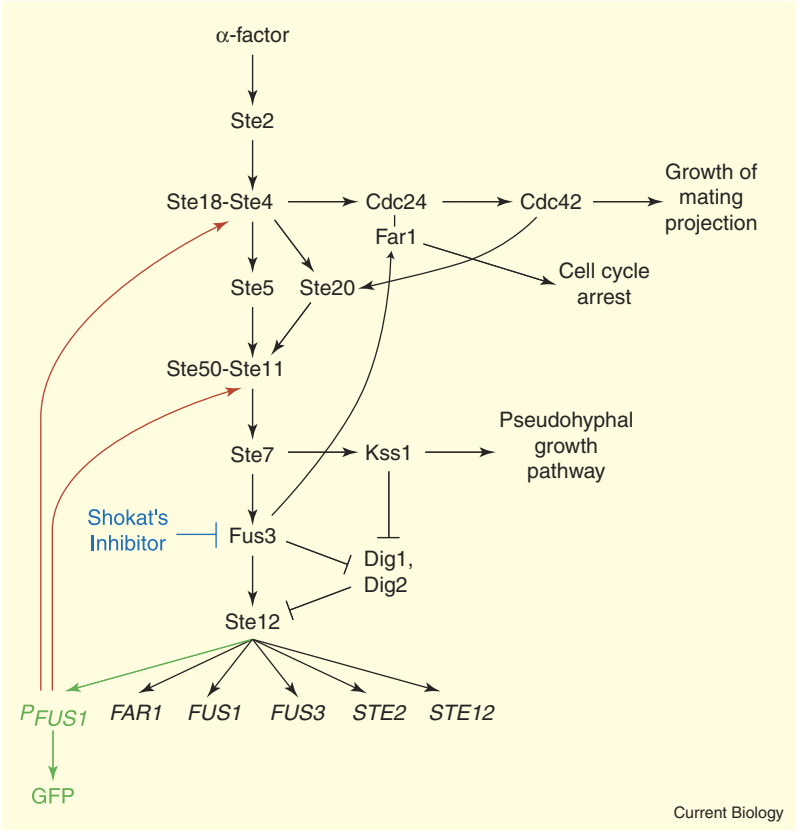

Figure 1. Yeast pheromone response signaling network.

Shown in black is the pheromone-response signal transduction network for wild-type yeast of mating type *MAT***a**. After α-factor is sensed by the Ste2 cell-surface receptors, the signal is transmitted through many steps until it arrives at the gene regulator Ste12; active Ste12 regulates expression from many genes, including those shown. The colored portions of the figure show some of the additions that are reported by Ingolia and Murray [3]. In green is an additional promoter for *FUS1*, which was made to express green fluorescent protein (GFP) as a reporter. This promoter was also used to make versions of Ste4 or Ste11, which added positive feedback, and thus bistability, to the reaction network (shown in red). Shokat's inhibitor, shown in blue, is a small molecule that was used in some experiments because it inhibits activity of a mutant form of Fus3.

This work is the latest in a series of papers backing the observation that it is relatively easy to rewire natural networks for new and interesting function. For example, a series of papers from the Lim lab [13] show that modularity in the scaffold-based recruitment of signaling molecules (in this same yeast pathway) can rewire the system for different input–output behavior, or can achieve the same behavior with multiple alternative scaffolds. A very recent paper by McClean *et al.* [14] showed that a mutual inhibition causes either the pheromone or the hyperosmolar response pathway to be active, but not both. A simple rewiring eliminated this switch-like behavior and allowed dual response. While all these examples

are drawn from yeast, and the pheromone pathway in particular, there are numerous studies in many organisms which together indicate that this evolvability is a rule rather than an exception.

These results support an emerging paradigm that sees the cell as a collection of hierarchical modules, the architecture of which has evolved for both robust function on time-scales shorter than evolution and ease of reconfiguration for new function on longer time-scales [1,15]. Apart from the profound implications on the evolvability of new function and even new organisms, there are also ramifications for the nascent field of synthetic biology. Synthetic biology seeks to make the engineering of complex function in microbes predictable, cheaper, scalable and more reliable [16] for applications spanning pharmaceuticals [17] to therapeutic bacteria and viruses [18,19]. If evolution has forced cellular designs to be modular and easily rewirable then it may be possible to exploit this design for new engineering purposes. The parsimonious routes to creating new switching, memory and controlled diversity with a natural pathway demonstrated by Ingolia and Murrary [3] lend further optimism to this program.

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Visual Neuroscience: Hypercomplex Cells in the Arthropod Visual System

Newly described visual interneurons in flies have sophisticated receptive field properties reminiscent of neurons in the mammalian visual cortex. The cells are well-suited to compute motion of conspecific females that male flies aerially intercept.

Cole Gilbert

"God in his wisdom made the fly, and then forgot to tell us why" or so wrote Ogden Nash. We do not know why flies evolved, but they serve as excellent subjects for research in visual neuroscience. In the 1950s, Werner Reichardt [1] began studying motion vision in insects primarily using house flies and blow flies. Unlike their smaller cousin, the fruit fly, these flies perform spectacular, visually guided aerial pursuits. Their ability to visually track and physically intercept a target at linear velocities of 2–3 meters per second and angular velocities of 3000–4000 degrees per second is simply amazing. How do flies with their quarter million visual interneurons [2] accomplish such feats? Reichardt's group developed the correlation model of motion detection, which quantitatively explains optomotor responses of flies in terms of whole animal behavior and cellular physiology [3] and is still one of the best network models in systems neurobiology. Big flies remain a vibrant model yielding exciting results as illustrated by the new work of Barnett *et al.* [4], published recently in *Current Biology*.

The new class of motion-sensitive neurons