Evolution of Novel Regulatory Behaviors

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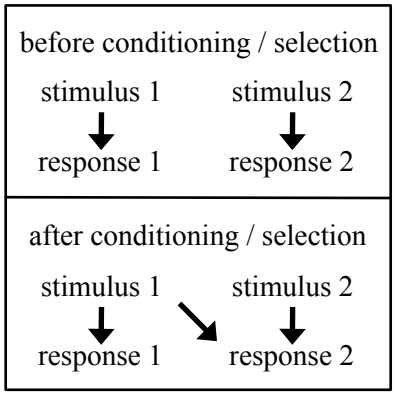
The ability to respond to changes in the environment is a defining feature of all organisms, which have evolved complex methods to regulate their physiology to match environmental variation. Direct regulation involves an organism first sensing a stimulus or a change in the environment and then mounting an appropriate response. However, the time required for sensing and responding results in a delay. Environmental variation at regular intervals selects for organisms that match their physiological regulation to the cycle. An example is the circadian clock, which regulates physiology in sync with the 24-hour day/night cycle. Recent work has demonstrated that, in environments where changes in two or more variables are coupled, a change in one variable can induce a transcriptional response for the other variable[1,2]. In other words, cells can encode information about correlations between environmental changes within their gene regulatory networks. This phenomenon has been termed "adaptive prediction" due to the fitness benefit of responding before rather than after an environmental challenge.

I propose to exploit this fitness benefit to select for cells that form a novel regulatory connection between a stimulus and a response (Aim 1). Abstractly, this scheme is analogous to classical conditioning in psychology, in which one stimulus (S1) is presented in temporal association with a second stimulus (S2) that elicits a response (R2) not induced by S1. After a period of conditioning, S1 elicits R2 (Figure 1). In animals, the connection between S1 and R2 occurs by associative learning within an individual; in single cells, this connection will be evolved via mutation and selection over many generations. I will identify causal genetic changes and characterize the molecular basis of the novel regulatory connection in the evolved strains (Aim 2). I will replicate the selection several times to determine whether multiple paths of evolution occur, and I will alter the selection conditions to determine how the path of evolution depends on certain parameters (Aim 3).

Figure 1

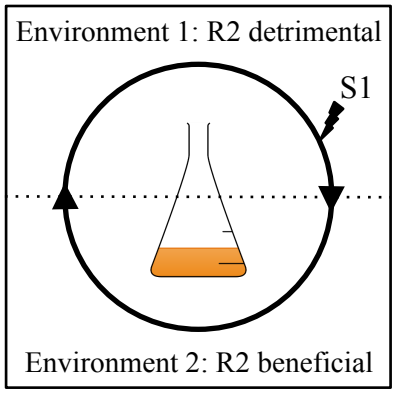


Aim 1: I propose to evolve a novel pathway of regulation in the single-celled eukaryote *Saccharomyces cerevisiae*. This will be achieved by selecting for a stimulus, S1, such as a defined change in an environmental variable (e.g. carbon source, temperature), to induce a previously unrelated response, R2 (e.g. pseudohyphal growth). A regulatory connection will be defined by the following criteria: (1) exposure to the stimulus induces the response; (2) the response is not induced in the absence of the stimulus. Here I outline the general experimental setup and a specific example where cell cycle arrest is R2 (Figure 2).

General setup: A large, log-phase culture of *S. cerevisiae* will be exposed to two alternating environments. In the first environment, E1, activation of a defined response (R2) will inhibit cell growth; in the second environment, E2, activation of R2 will promote cell survival. At a defined time prior to switching from E1 to E2, cells will be exposed to the conditioning stimulus (S1). Cells that induce R2 upon exposure to S1 will have a significantly greater chance of survival in E2. The strongest selection will kill non-responding cells in E2. The counter-selection provided by the need to proliferate in E1 will prevent cells from utilizing other strategies, such as constitutive or stochastic activation of R2, to pass the selection. The yeast strain used will possess an elevated mutation rate to increase genetic diversity in the population.

Specific scheme: This scheme exploits the survival benefit of cell cycle arrest in certain environments to select for a novel induction of arrest (R2) by a stimulus. A large, log-phase culture of cells will be transferred from rich medium, in which cell cycle arrest is selected against, into a medium containing hydroxyurea (HU), a DNA replication inhibitor. Proceeding through mitosis with unreplicated DNA kills cells. Wild-type cells avoid this fate by triggering the S-phase checkpoint to halt the cell cycle in the presence of HU; however, this checkpoint is disabled in certain mutants such as *mec1Δ*[3]. Conducting the selection with checkpoint-defective cells selects for cells that can arrest the cell cycle via another mechanism. Before transfer into HU medium, cells will be exposed to a high copper concentration (S1). Excess copper induces activation of *CUP1*, which encodes a metallothionein that sequesters copper ions to protect against toxicity (R1)[4]. Because cell cycle arrest prevents proliferation in rich medium, the optimal strategy is for cells to arrest the cell cycle upon exposure to increased copper and resume the cell cycle when restored to trace amounts of copper.

Figure 2



Aim 2: I will determine the specific genetic changes that give rise to the new regulatory connection and characterize the connection at the molecular level. The Murray lab uses experimental evolution in several projects and has developed a pipeline to identify causal mutations in evolved yeast strains. This expertise will aid me in characterizing the strains that are produced in Aim 1. The molecular basis of the novel connection could be the generation of crosstalk between two existing signaling pathways by a change in transcription factor binding or kinase specificity. For the specific selection described above, I predict that the activity of the Cup2 protein (also known as Ace1), the copper-responsive transcriptional activator of *CUP1*, may be altered to impinge upon one of the many pathways that can induce cell cycle arrest.

Aim 3: I will perform replicates of each scheme to investigate the occurrence of different evolutionary solutions to the selection imposed. I can control several parameters of the selection: the percent survival of non-responding cells in E2 (i.e., the strength of the selection), the amount of time spent in each environment, how long S1 is present, and the time interval between S1 and the switch to E2. By altering these variables, I will investigate how the details of the selection conditions affects the speed and path of evolutionary changes.

Broader Impacts: Studying how selective pressures create novel phenotypes is crucial to understanding and combating emerging diseases, resistance to antibiotics and antiviral therapies, and cancer resistance to chemotherapy. In addition to providing insight into evolutionary mechanisms, my proposed research can play an important role in demonstrating to non-scientists the power of selection in determining the evolutionary trajectories of organisms. Despite the overwhelming evidence of a common ancestry for living organisms, 40% of the U.S. population denies that evolution has shaped the human species[5]. This huge gap between accumulated scientific knowledge and the public level of understanding is detrimental to U.S. society. This is why I am committed to science education, both through direct interactions, such as teaching and tutoring, and by making the results of scientific research, including my own, known to a general audience through modern social media (see Personal Statement).

References: [1] Tagkopoulos *et al.*, Science 2008; [2] Mitchell *et al.*, Nature 2009; [3] Weinert *et al.*, Genes Dev. 1994; [4] Butt *et al.*, PNAS 1984; [5] Gallup Poll. Dec. 10-12, 2010. N=1,019 adults nationwide, Margin of error ±4%