Title: Gratuitous induction of *FAR1-22* to mediate cellular arrest by both blue light and β-estradiol and characterization of stress due to blue light and Cry2 and Cib1 in *S. cerevisiae*.

Goal: To characterize the sensitivity of the cell cycle to Far1-22p, create a blue light or β-estradiol inducible construct to promote cell arrest in *S. cervisiae*, and quantitatively assess the response of yeast to blue light and Cry2 and Cib1.

Background and significance: The current set of tools to induce cell cycle arrest and synchronize cells for study of the mitotic cell cycle or of related pathways are limited, expensive, and non-gratuitous, either activating unrelated genes or damaging the cell. One such technique relies on the fact that entry from the G_1 to the S phase of the cell cycle is mediated by Cdc28-Cln kinase, can be inhibited by Far1p upon activation of the mating pathway (1). While overexpression of *FAR1* alone does not cause cell arrest, as the protein must be post-translationally modified by other components of the pathway, a dominant *FAR1* mutant, *FAR1-22*, has been shown to arrest the cell in G_1 when expressed (2). As Far1-22 is missing a site necessary for Cdc28-Cln triggered degradation, it is particularly stable and prevents the relocation of Cdc24, a factor required for cell polarization, to the cell membrane (7).

I propose the use of a gratuitous inducer to express *FAR1-22* and quickly and efficiently induce cell cycle arrest. This would provide an unparalleled tool to synchronize cells and study the cell cycle and related pathways. I will use two methods to singly express *FAR1-22*. Using a well characterized zinc finger promoter, I will regulate *FAR1-22* using a β-estradiol regulated system which relies on the estrogen receptor to only localize the transcription factor construct to the nucleus in the presence of inducer (6). Similarly, using the same promoter, I will separately induce the cell cycle gene with a transcription factor utilizing a set of *Arabidopsis* proteins Cry2 and Cib1 (8), which only interact in the presence of blue light (3). Both approaches permit sensitive, fast, and nearly gratuitous transcriptional regulation of single genes in *S. cerevisiae*. In order to provide a more robust basis for this tool, I also propose to analyze the effects of blue light and the toxicity of the Cry2 and Cib1 proteins to *S. cerevisiae*.

Aim 1: *Evaluation of yeast cell cycle sensitivity to Far1-22p.* In order to characterize the sensitivity of growth to the level of Far1-22p, I will transform *S. cerevisiae* cells with plasmids containing *GAL4* promoters in front of *FAR1-22* (from D. Botstein) and *FAR1* (control, to be built by homologous recombination in yeast). The sensitivity of the cell cycle to the level of *FAR1-22* will be assessed quantitatively by first growing cells (*FAR1-22*, *FAR1*, and *WT*) overnight in raffinose and then taking serial dilutions of the original colony and measuring colony forming ability on plates with 2% galactose and $0 - 2\%$ glucose at 0.25% intervals. This will permit me to determine the appropriate level of induction by β-estradiol or blue light. In order to ascertain whether cells exit the G1 phase at the same time once *FAR1-22* is turned off, the addition of glucose will be used to turn off *FAR1-22* expression in cells grown in galactose and minimal glucose. These cells will then be stained with propidium iodide to determine DNA content over time using flow cytometry. If time permits, the rate at which cells exit the G1 phase of the cell cycle after α-factor mediated cell arrest will also be calculated using flow cytometry as a comparison to *FAR1-22*. Furthermore, the effect on growth rate of the *FAR1-22* and *FAR1-WT* strains will be assessed by growing cells overnight in raffinose and measuring OD_{600} over time of cells at varying concentrations of

glucose and galactose.

Aim 2: *Construction of a system to gratuitously induce cell arrest in yeast.* I will knock out *TRP1* with the CORE cassette (composed of *URA3* and *KANMX*) from the pCORE plasmid in a previously engineered strain containing the ZEV system. After selecting for G418 and FAA resistance and 5-FOA sensitivity, I will then replace this cassette with a zinc finger promoter corresponding to the zinc finger DBD and *FAR1-22* (and *FAR1* as a control) and select for this recombinant by testing for 5-FOA resistance and G418 sensitivity. Once the strain has been sequenced to confirm the replacement, I will induce *FAR1-22* with differing levels of β-estradiol (1 to 100 nM) and test colony forming ability as outlined in *Aim 1* (6). Given that this strain does not show any growth defect and demonstrates cell arrest comparable to that seen in *Aim 1*, I will transform the strain with plasmids containing the ZiF_{268} *DBD-CRY2* and *GAL4 AD-CIB1* constructs (plasmids from M. McClean). After confirmation of plasmid sequence, transformed yeast will be exposed to a range of fluence rates of blue light (from 42 to 120 20 mmol m^2 s^1) and cells will be visualized by microscopy to confirm cellular arrest. The sensitivity of growth to the level of Far1-22p will be assessed quantitatively by measuring OD_{600} of cells grown at various light intensities and qualitatively by the rate of colony formation on plates with minimal media (in glycerol). The long lifespan of Far1-22p relative to Far1p ($\sim$ 120 rather than $\sim$ 30 minutes) may cause cells to exit G1 in an unsynchronized manner. If flow cytometry demonstrates this to be the case, I will construct a version of Far1-22p with a shorter lifetime by placing a sequence for an N-degron tag at the front of *FAR1-22* (5). Furthermore, it may be possible to degrade Far1-22p by expressing non-localized cdc4, which degrades Far1p in *WT* (4).

Aim 3: *Characterization of yeast response to CRY2 and CIB1 induction under blue light.* To assess the impacts of using blue light to express genes with Cry2/Cib1 I will investigate the effect of blue light on yeast. Growth rate and gene expression will be measured at a range of fluence rates of blue light (from 42 to 120 20 mmol m^2 s^1) in *S. cerevisiae* cells transformed with the plasmids containing the constructs under the ACT1 promoter. I will measure growth using OD_{600} readings and perform microarrays for the fluence rates with measured growth rate defects of more than 2%, using uninduced samples as control. To analyze microarray data, I will cluster genes by expression and search for affected pathways and potential binding sites to the Cry2/Cib1 construct. If growth defects are not correlated with gene expression, I will measure growth with Cry2 and Cib1 expressed under weaker constitutive promoters to test for toxicity.

Expected Outcomes: Upon completion of my aims, I will have characterized the sensitivity of the cell cycle to Far1-22p expression using the GAL promoter. Furthermore, I will have created a gratuitous system for inducing cell arrest using either blue light or β-estradiol. If experimentation using the GAL promoter reveals that I have to shorten the lifespan of Far1-22p due to its interaction with Cdc24, I will do so by tagging it for degradation. I will also have characterized the impact of the *CRY2-CIB1* system and blue light on yeast.

Broader Impacts: Completion of my aims will not only lead to the creation of a simple and efficient method for cell synchronization to be used in work on cell cycle related networks, but my analysis of the optogenetic transcription factor system will inform use of this tool in future single gene studies, in particular for network analysis. Continuing experimentation could focus on the creation of a switch-like signal for the light induced constructs by placing the constructs under their own promoters. The creation of a bistable switch-like system for

using the transcription factor would also add to the possibilities for network study. Finally, the full system could be placed on to one or two plasmids in order to conveniently enable cell arrest in any strain.

References:

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