Abstract

When engineering a biological system, it would be useful to have switchable control for all steps of gene expression, from transcription to protein-translation modification. These switches should not only be fast but also reversible. We design a system that incorporates temporally controlled protein-protein interactions. This project has two components. We are originally used as a transcriptional regulator, to control translocation and targets within the cell by varying light conditions. Under exposure to red light, a protein of interest localizes to a particular target, while exposure to far-red light causes the protein to diffuse throughout the cell. This is accomplished using PhyB, a phytochrome from plants, and PIF3, a transcription factor within the phytochrome band. We are using this system, originally used as a transcriptional regulator, to control translocation and protein-protein interactions. This project has two components. We are metabolically engineering yeast to produce PCB, a chromophore component of PhyB. Additionally, we are using the PhyB-PIF3 switch mechanism to control the translocation of a protein of interest part of the cell.

System Overview

- s.cerevisiae is engineered so that light can be used to give spatial and temporal control over a protein of interest through a quick, reversible bioswitch.
- When pulsed with red light, a protein of interest rapidly localizes to a specified target within the cell.
- When pulsed with far-red light, the protein diffuses throughout the cell.
- All switch components are produced endogenously in the cell.

Mechanism adopted from plants & algae

- In red light, phytochromes switch from their inactive Pr conformation to its active Pfr conformation.
- In far-red light, phytochromes switch back from the active Pfr conformation to their inactive Pr conformation.

PhyB-PIF3 bioswitch

The chromophore for the phytochrome PhyB is phycocyanobilin (PCB) in cyanobacteria and phycobilisome (PBS) in plants. PhyB binds to PIF3, a phytochrome interactive factor, when activated by red light to its Pfr conformation.

PCB Purification:

- PCB is purified from Spirulina powder, a dietary supplement made of the cyanobacteria Arthrospira.
- Absorption spectrum of product met our expectation of high absorption at 680 nm.

PCB Synthesis Pathway

- Overexpression of HMXX
- PhyB is an enzyme which converts biliverdin to PCB, found in cyanobacteria.
- PhyB was synthesized by GeneArt and cloned into it a high-copy plasmid with a constitutive ADH1 promoter.

Part 1: Metabolic Engineering

Overview:

- We are engineering yeast to produce PCB endogenously.
- Phycocyanobilin (PCB) is essential for the PhyB-PIF3 system to work.
- PCB biosynthesis has two steps: converting heme to biliverdin and biliverdin to PCB.
- PCB absorbs red light (660 nm) in its Pr form and far-red light (720 nm) in its Pfr form.

Part 2: Protein Localization

Overview:

- Our system utilizes an anchor-traveler mechanism to localize proteins.
- Either PhyB or PIF3 functions as an anchor by being constitutively localized to the target site in the cell.
- The other protein, the traveler, is bound to a protein of interest and navigates the cell via diffusion.
- The wavelength of light present controls whether or not the traveler binds to the anchor.
- In red light, PhyB switches to the Pr conformation and associate with PIF3. The traveler associates with the anchor and localizes the protein of interest to the target.
- Far-red light causes PhyB and PIF3 to dissociate. The traveler can again diffuse throughout the cell.

Methods:

- Method 1: Sequential Cloning
  - PhyB and CFP were inserted sequentially into a pRS313 vector with a constitutive promoter, as were PHYB and YFP.
- Method 2: Homologous Recombination
  - Each of the DNA sequences carried a 40 bp homologous region.
  - Anchor constructs include signal sequence for localization.
  - PIF3 tagged using N-terminal signal sequence.

Results and Next Steps:

- Plasmids with PhyB-CFP and YFP-PIF3 were constructed and sequenced.
- Sequence results showed that expected constructs were built.
- Plasmids were transformed into yeast and observed using fluorescent microscopy.
- Potential causes for lack of expression should be tested and the constructs re-built.
- Signal sequences should be added to complete our anchor and traveler constructs.

System Advantages

- Driven by light, which is cheap and easy to produce.
- No need for exogenous chemicals, which can be difficult to remove from a system once induced.
- Orthogonal and self-contained system.
- Strong spatial control via laser pointer.
- Very fast temporal response on order of seconds for binding.

Applications

1. Mitotic and meiotic synchronization without addition of drug molecules.
- Sequestration cell cycle regulators from their intended compartment to arrest cell cycle.
- Release from cell cycle arrest for synchronized growth without washing cells.
- Localize proteins to an organellar.
- Release proteins at same time and wash diffusion throughout the cell.
3. Temporal and spatial protein regulation in cells grown on solid surface.
- Control protein activity in designated sectors within fungal colonies and biofilms.
- Study biofilm formation by pathogenic fungal species.

Part 1: Metabolic Engineering

PCB Biosynthesis Pathway

- Overexpression of HMXX
- PhyB is an enzyme which converts biliverdin to PCB, found in cyanobacteria.
- PhyB was synthesized by GeneArt and cloned into it a high-copy plasmid with a constitutive ADH1 promoter.

Part 2: Protein Localization

Design:

- Two Anchor-Traveller Systems were designed for Protein Localization.
- PhyB is fused with CFP and PIF3 with YFP for visualization using fluorescent microscopy.
- Anchor constructs include signal sequence for localization.
- PhyB tagged using C-terminal signal sequen.
- PIF3 tagged using N-terminal signal sequence.
- Signal sequences target nuclear membrane, plasma membrane, mitochondria, and mitochondrial membrane.

Methods:

- Method 1: Sequential Cloning
- PhyB and CFP were inserted sequentially into a pRS313 vector with a constitutive promoter, as were PHYB and YFP.
- Method 2: Homologous Recombination
- Each of the DNA sequences carried a 40 bp homologous region.
- Anchor constructs include signal sequence for localization.
- PIF3 tagged using N-terminal signal sequence.

Results and Next Steps:

- Plasmids with PhyB-CFP and YFP-PIF3 were constructed and sequenced.
- Sequence results showed that expected constructs were built.
- Plasmids were transformed into yeast and observed using fluorescent microscopy.
- Potential causes for lack of expression should be tested and the constructs re-built.
- Signal sequences should be added to complete our anchor and traveler constructs.

Acknowledgements

Graduate Advisors

- Brian Belmont
- Chia-Yung Wu
- Stephen Goldfeder
- Scott Carlson
- Kari silverFeld
- Ina Kiyoko

Faculty Advisors

- Prof. Alexander Molchanov
- Prof. Lisa DeFazio
- Prof. Nadia Kabbadi
- Prof. Katarzyna Prather
- Prof. Gregory swimmington
- Prof. Jimi Lee
- Prof. Soo Hee Moon

References