Engineering optogenetic actuators

UV Light-controlled gene expression in Plants

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Abstract

Controlling gene expression of plants at both the single cell and whole organism level with temporal resolutions as good as 10 minutes and minimal off-target effects. All this can be achieved by using a gene expression system controlled by light[1]. To this end we are re-engineering the UV responsive UirR/S two-component system from the cyanobacterium Synechocystis PCC6803 for use in plants. All you will have to do is expose the plant to UV light and expression of your gene of interest is turned on, then expose the plant to green light and the system is turned off. We have established the system's compatibility with the plant chromophore PΦB by cloning of different chromophore synthesising constructs and expression in E.Coli. This makes the UirR/S system a promising candidate for further development.

Background

Two component systems are the foremost way in which bacteria sense and respond to their environment.[2] They consist of a membrane bound sensor histidine kinase and a DNA binding response regulator. The UirR/S system controls expression from the P_{csiR1} promoter in the response to UV and green light. UV light turns on gene expression, by activating auto phosphorylation of UirS, which allows for phosphotransfer to the promoter binding UirR response regulator. Green light turns off gene by promoting dephosphorylation of UirS. This system was adapted for expression in E.coli by Ramakrishnan and Tabor (2016)[1](Figure 1).

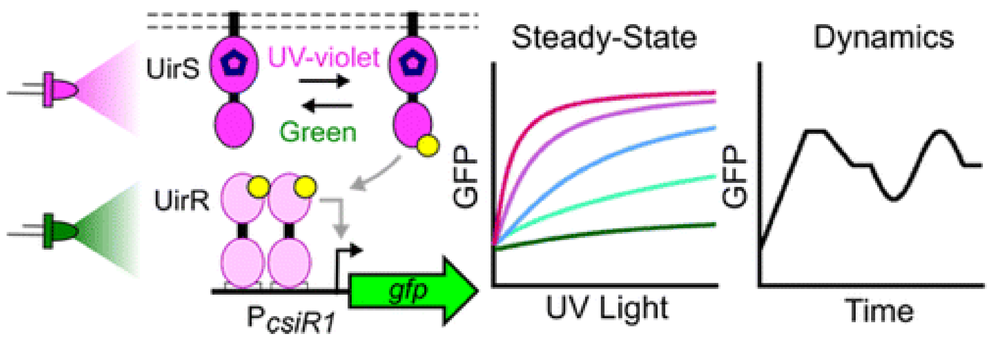

Figure 1: Schematic of the UirR/S system Ramakrishnan and Tabor (2016)1

Objectives

- We are attempting to re-engineer the UirR/S system into a light controllable gene expression system with minimal off-target effects and unrivalled spatiotemporal resolution.
- The system will be activated by UV light and remain inactive under ambient light conditions.
- In plants we want UirR/S to function using the plant chromophore phytochromobilin (PΦB).

Testing the chromophore dependence of the UirR/S light inducible expression system.

Testing the chromophore dependence of UirS: In its native cyanobacterial host UirS binds to the chromophore phycocyanobilin (PCB). While this chromophore is not produced in plants, a very similar chromophore phytochromobilin (PΦB), is produced (Figure 2). We therefore set out to investigate the behaviour of the UirR/S system in the presence of these two chromophores, their common precursor biliverdin (BV) as well as in the absence of any chromophore. We used In-Fusion cloning, a next generation cloning technique that allows for seamless construct design, to create plasmids expressing genes involved in the synthesis of these chromophores. Constructs were expressed in E.Coli. and incubated in the presence of different artificial light sources (UV, White, Green, Dark). We optimised expression levels from the system by engineering the -10 site of the P_{csiR1} promoter and the ribosomal binding site of the sfGFP reporter gene. We were able to show that the UirR/S system behaves in the same way when UirS bind the plant chromophore PΦB as when it binds its native cyanobacterial chromophore PCB. (Figure 3)

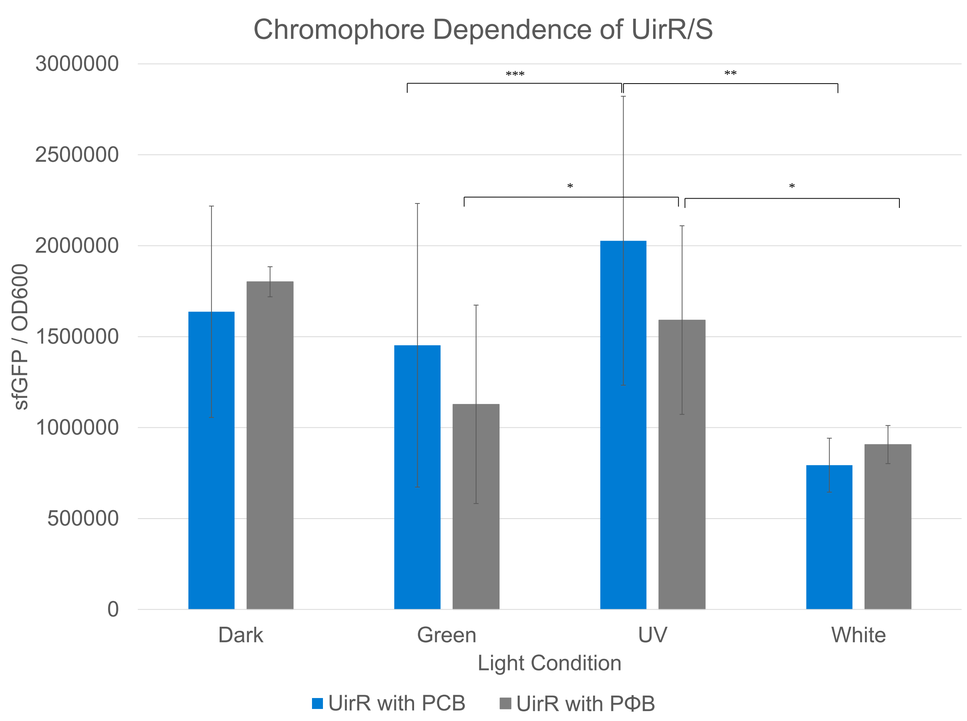

Figure 3: Chromophore Dependence of UirR/S system, error bars represent standard error, n=6

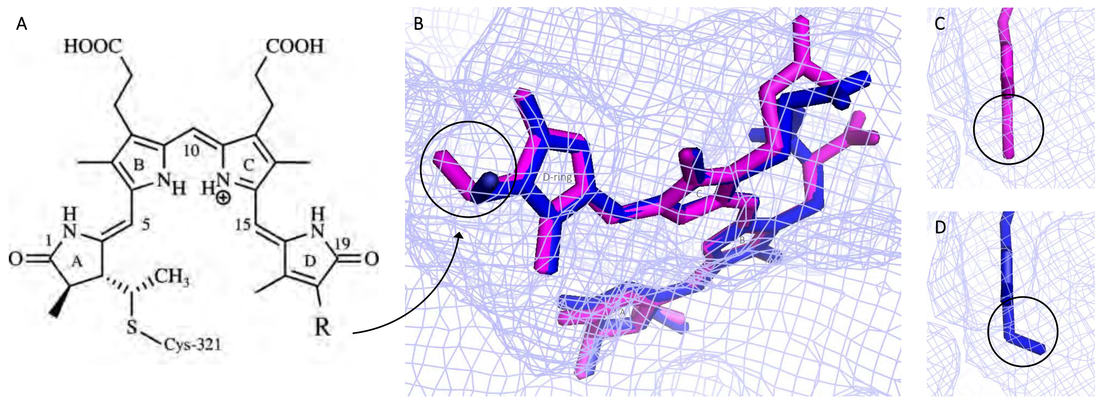

Figure 2: A Phytochromobilin, R=vinyl, and phycocyanobilin, R=ethyl. **B** Superimposition of phytochromobilin (magenta) and phycocyanobilin (blue) in the chromophore binding GAF domain of the green/red cyanobacteriochrome, AnPixJ[3]. **C&D** Side views of the of phytochromobilin and phycocyanobilin D-rings.

Perspectives

- The UirR/S system is a promising candidate to be further developed into an optogenetic system for plants. This involves making UirS soluble and unclearly localised to enable efficient gene activation through UirR.
- The fully developed system will allow for precise control of gene expression in plants, allowing for novel experimental design. Among other things it could modulate complex signalling networks such as phytohormone concentrations, or be used in agricultural applications to control the plant life cycle.

1. Prabha Ramakrishnan and Jeffrey J. Tabor. (2016,). Repurposing Synechocystis PCC6803 UirS–UirR as a UV-Violet/Green Photoreversible Transcriptional Regulatory Tool in E. coli. *ACS Synth. Biol.*. 5 (7), p 733–740.
2. Gao,R.,andStock,A.M.(2009)Biologicalinsightsfrom structures of two-component proteins. Annu. *Rev. Microbiol*. 63, 133–54.