A Genetic Toggle Switch in Arabidopsis

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***Short Abstract* — Plant synthetic biology has tremendous potential, but the complexity of plant biology has made it difficult to engineer complex synthetic circuits in plants. Here we report the construction of the first genetic toggle switch made in a plant. We used a forward engineering approach by first analyzing synthetic repressors and cognate promoters in plant protoplasts. Based on in silico predictions arising from this analysis, we then selected combinations of repressors that could work as a toggle switch, assembled and inserted them in a plant, using luciferase as a reporter for switch performance. Mathematical analysis of the results led to the conclusion that one of the two combinations satisfied the tests for bistability.**

***Keywords* — Synthetic Biology**

I. PURPOSE

Synthetic biology has made immense strides in the development of programmable genetic circuits in bacteria and mammalian cells, yet the development of complex synthetic circuits in plants has lagged behind. Despite a long history of plant genetic engineering, the complexity of plant biology appears to have slowed the development of synthetic circuits in plants. However, plant synthetic biology is of great interest since it can potentially lead to sustainable green technologies for human needs.

The basic toolbox of synthetic biology comprises of switches, oscillators, and logic gates. A genetic toggle switch, which translates analog external stimuli into binary internal outcomes, was one of the earliest information-processing synthetic circuits developed in E. Coli in 2000. Toggle switches of varying architectures are known to operate in multiple developmental processes in plants. The development of a synthetic toggle switch in plants could have a significant technological impact because it would allow us to exogenously control plant traits.

Motivated by the above arguments, we set out to develop a toggle switch in plants. Here we report on results from a collaborative effort to develop the first synthetic toggle switch ever developed in a plant.

II. TESTING GENETIC PARTS

A key step in the rational design of synthetic networks is the quantitative characterization of components to enable predictive modeling. This poses special difficulties for plants, since stably transforming plants is time consuming. We developed an experimental system for rapid quantitative measurements of synthetically designed repressors using plant protoplasts but found that protoplast assays show significant experimental batch effects that lead to incorrect quantitative results. With the help of a mathematical model coupled with stochastic simulations, we were successful in explaining and normalizing the batch effects to make quantitative comparisons between different inducible repressors, and approximately predict quantitative properties of synthetic circuits in stably transformed plants. We tested hundreds of repressible promoters and carried out a statistical analysis of the quantitative data to uncover design principles for building synthetic inducible repressors in plants.

II ASSEMBLING THE FULL TOGGLE

Quantitative analysis of the genetic parts yielded quantitative parameters, that we could use to simulate a mathematical model of the system and predict the combinations most likely to work in the plant. This quantitative analysis led to the discovery that many repressible promoters were very different in basal expression and sigmoidality properties. Guided by this analysis, we chose two combinations to test, assembled and inserted them in Arabidopsis and used luciferase luminescence to report on circuit behavior. Assessing circuit behavior in plants was affected by unexpected phenomena such as tissue specific differences and the confounding effects of plant growth. To test for bistability we constructed a suite of independent experiments examining different behaviors of the toggle, and then fitted the resulting experimental data to a mathematical model of the toggle switch. We sampled parameter space of good fits using MCMC sampling. Analysis of the results led to the conclusion that one of the two combinations satisfied the tests for bistability. We have thus successfully made the first plant toggle switch!

III. CONCLUSION

We report here the construction of the first genetic circuit in plants – a genetic toggle switch in Arabidopsis.

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