Control of the *GAL* Network in *S. cerevisiae* for Use in Biological Control Theory

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***Short Abstract* - The complex network of control mechanisms associated with biological systems provides an immense challenge to understanding these systems. In order to characterize the dynamic cellular functions within these complex systems, the system's real-time response to specific perturbations must be analyzed. This project uses the GAL network within *Saccharomyces cerevisiae* as the biological system of choice for demonstrating control via extracellular signaling molecules. A strain of yeast with the GAL1 gene tagged with Venus fluorescent protein is used so that the dynamic response of gene expression can be monitored by measuring real-time fluorescence intensity.**

I. PURPOSE

The intricate network of controls associated with biological systems adds great complexity to the challenge of fully understanding them. In order to characterize the dynamic cellular functions within these complex systems, the system's real-time reactions to specific changes must be analyzed. Our project utilizes the budding yeast *S. cerevisiae* to serve as a model for eukaryotic systems.

Although control of biological systems is a growing field, there is little work being done on the control of the external regulation of cellular functions. This project uses the *GAL* network within *S. cerevisiae* as the biological system of choice for demonstrating control via extracellular signaling molecules due to how well-understood it already is. In order to avoid compromising the complexity of the *GAL* network, we use a microfluidic multitrap nano- physiometer platform in order to monitor the response of single cells. We aim to predict control of the system by first obtaining data on the response of the *GAL* network to extracellular signals, which can then be run through a mathematical model reduced from the *GAL* network [1].

Specifically, we have monitored the dynamic response of gene expression within the *GAL* network to changes in the carbon source of the media by switching between raffinose, galactose, and glucose as carbon sources. By using a strain of yeast in which the *GAL1* gene has been tagged with the fluorescent protein Venus, the response can be monitored by measuring the real-time fluorescence intensity of the yeast cells, which indicates changes in gene expression levels [2].

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II. RESULTS AND CONCLUSION

When yeast, grown overnight in glucose media, were induced by the presence of galactose in the media, the *GAL1* promoter was successfully induced. No average increase in fluorescence can be seen in the first 60 minutes, which is in agreement with the fact that the media was not switched from SC + 2% raffinose to SC + 2% galactose until after 60 minutes. From its uninduced state in raffinose media, the *GAL1* promoter in the budding yeast *S. cerevisiae* was induced by galactose, triggering the transcription and subsequent production of the Venus fluorescent protein, leading to an increase in fluorescence. Once the media was switched to the SC + 2% galactose media, the average fluorescence detected steadily increased until reaching a maximum peak around 400-450 min.

In the galactose induction followed by raffinose uninduction experiment, the *GAL1* promoter was successfully induced by galactose and then allowed to uninduce by switching back to raffinose media. When the media is switched back to raffinose, however, fluorescence continues to increase. This most likely reflects the fact that raffinose does not actively repress the GAL Network, and therefore it takes time for the cellular machinery activated by galactose to diffuse and stop working.

In the glucose repression experiment, the *GAL1* promoter, which had been fully induced overnight, was repressed by glucose, preventing the transcription and subsequent translation of the GAL genes. No average decrease in fluorescence can be seen in the first 60 minutes, which is in agreement with the fact that the media was not switched from SC + 2% galactose to SC + 2% glucose until after 60 minutes. Upon the introduction of glucose, a general decrease in fluorescence can be seen. The active repression accounts for the relatively faster decrease in fluorescence compared to previous experiments.

REFERENCES

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