479f Dynamics of Cell Populations Carrying Gene-Switching Networks with Fluorescent Protein Markers of Different Half-Lives

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The impressive recent advances in molecular biology, genomics, transcriptomics, and proteomics have provided us with powerful tools for studying individual processes at the cellular and molecular levels. However, biological behavior is not only a result of interactions among the components within individual cells; it also strongly depends on the complex, direct and indirect interactions among the cells of a population. These intra- and inter-cellular interactions lead to significant phenotypic cell-to-cell variability, or cell population heterogeneity. Since the phenotype of each individual cell is strongly influenced by the function of genetic regulatory networks, cell population heterogeneity is tightly related to the architecture of such networks. Hence, in order to be able to rigorously design gene regulatory networks for biotechnological applications, we first need to understand the relationship between the characteristics of networks with specific genetic architectures and the distribution of phenotypes amongst the cells of the population.

The study of model synthetic regulatory networks offers the opportunity to give insight into the behavior of naturally occurring networks as well as to provide a platform for expression control for technological applications. The model regulatory network under investigation here is a toggle switch composed of two coupled inducible promoter-repressor pairs. An existing plasmid with a toggle network and a green fluorescent protein (GFP) reporter gene [1] was first inserted into an E. coli host cell population. The GFP levels were monitored over time with a flow cytometer for shake flask cultures, until balanced growth was reached. These measurements were taken at a wide range of inducer levels, and they allowed for characterization of the average behavior of the system as well as the distribution of fluorescence among the cells of the population. For lower inducer concentrations, the system exhibited unimodal GFP distributions with monotonically increasing mean expression levels, while for higher inducer concentrations the unimodal distributions were practically aligned around the same mean. On the contrary, for intermediate inducer concentrations, the distribution was found to be bimodal, while the dynamics until the system reached balanced growth were noticeably slower than those for very low or very high inducer concentrations. We further constructed a modified genetic toggle network where the GFP marker has a shorter half-life and the modified plasmid was inserted in the same host cell population. Detailed characterization of this construct with the use of flow cytometry provided insight into the effect of the GFP degradation rate on the aforementioned balanced growth and transient distribution patterns as well as on the average expression dynamics. To obtain further insight into the behavior of this bistable genetic network, we employed a mathematical model describing the genetic toggle and GFP expression dynamics. Through comparison with the experimental data, the model results offered explanations of the experimentally observed transient and balanced growth patterns.

[1] Gardner, T.S., C.R. Cantor, and J.J. Collins, Construction of a genetic toggle switch in Escherichia coli. Nature, 2000. 403: p. 339-342.